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A SPECIES OF RHIZOPHIDIUM PARASITIC ON THE OOSPORES OF VARIOUS PERONOSPORACEAE[1]

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WITH PLATE IV

After a method had been worked out for germination of the asexual spores of the Peronosporaceae (9), attention was directed to the germination of the sexual spores, oospores, of the above family. The necessity of studying this problem is at once apparent when we consider that the oospores of only a few species of this group have been germinated and, still further, when we recall that, since the work of De Bary (6) in 1863, practically nothing has been added to our knowledge on this subject.

In my studies of oospore germination, considerable difficulty has been encountered due to a parasite, *Rhizophidium pollinis*, which attacks the ripe oospores when they are placed in water to germinate. Similar difficulties have been reported by De Bary (6) in 1863 and Schroeter (11) in 1877, though none of the other numerous investigators of this problem report such experiences.

De Bary (6) states that considerable difficulty was experienced in germinating the oospores of Cystopus due to species of Mucor and Chytridium infesting them. In his later work, however, no further reference is made to either of these parasites. Schroeter (11) attempted to germinate the oospores of *Protomyces graminicola*, now known as *Sclerospora graminicola*, but did not succeed, although many tests were made both in water and on moist blotting paper. He states that the previously contracted protoplasm of the oospore took on a granular appearance, and later divided into eight to twelve elliptical bodies. No further development resulted, however, and Schroeter was not sure whether this appearance was due to swarm spore formation or simply the disintegration of the protoplasm due to its death. At this point the author adds the following footnote regarding a parasite that infested the oospores.

[1] This account is based on work done in the Plant Pathological Laboratories at the University of Wisconsin from 1910 to 1912. My sincere thanks are due Professors Jones and Harper for helpful suggestions during the progress of the work.

"Auf den auf Wasser ausgesäten Sporen fand sich ein Chytridium ein, das bald so überhand nahm, dass seiner Ausbreitung vielleicht die Misserfolge der Culturen zuzuschreiben waren. Diese Chytridien sassen frei ohne Spur einer Wurzelbildung auf der Oberfläche der Sporen, einzeln oder gesellschaftlich, oft in grosser Menge, zu 10 und mehr an einer Spore, nur an einem Punkte angeheftet un leicht ablöslich. Sie sind kuglig, nur an der Spitze manchmal schwach kegelförmig zugespotzt, bei der Reife 17 bis 24 Mikr. im Dchm. Die Ausbildung und Entleerung der Schwärmsporen erfolgt besonders des Morgens mit grosser Lebhaftigkeit. Bei der Entleerung öffnete sich die Chytridiumzelle mit einer einzelnen flachen oder nur schwach kegelförmig vorgezogenen Oeffnung. Die Schwärmsporen sind kuglig, etwa 3 Mik. im Dchm., mit einem farblosen Oeltropfen versehen.

"Der Parasit scheint von *Phlyctidium pollinis* A. Br. morphologisch nicht verschieden zu sein. Auf den in diesem Frühjahr wieder ausgesäten Protomyces-Sporen von 1877 hat sich das Chytridium wieder in derselben Menge eingestellt."

Although this description is brief and somewhat inaccurate, there can be no doubt as to the fungus Schroeter had under consideration. That it may become abundant and is a menace to the study of oospore germination as reported by De Bary and Schroeter is borne out by my experience. This fact, coupled with its imperfect description and morphology, makes it seem desirable to record some of my studies of this parasite.

Rhizophidium pollinis was probably first observed by Itzigsohn of Switzerland in 1847. It was found on *Conferva bombycina* and named by him *Chytridium vagans*, but apparently not described. Later Braun (3) described very briefly a *Chytridium pollinis Pini* infesting the pollen of *Pinis sylvestris* which he believed to be the same species as found by Itzigsohn on *Conferva bombycina*. Braun's description is briefly as follows:

"*Ch. pollinis Pini*. Eine gleichfalls kugelige Art von kaum über 1/50 mm. Durchmesser, welche sich unregelmässig mit einer einzigen grossen Mündung offnet. Die schwärmenden Keimzellen fast kugelig 1/400 mm. lang. Die Haut dieser Art ist dicker, als bie den verwandten und der Inhalt zeigt in der Jugend einen sehr grossen, erst spät verschwindenden Öltropfen. Auf in's Wassen gefallenen Pollenkörnern von *Pinus sylvestris* bei Berlin Anfang Juni d. j. gefunden."

In 1857 Schenk (10) established the genus Rhizophidium and later Fischer (8) modified the characters of the genus so that it included the genera Phlyctidium, Sphaerostytidium, Rhizophidium, and Rhizophyton of Schroeter. Zopf (12) referred *Chytridium pollinis Pini* to the genus Rhizophidium, giving the species as *R. pollinis*.

Method. Attempts were made to germinate the oospores of *Cystopus*

bliti, found in the leaves, stems, and flowers of *Amaranthus retroflexus* which grow abundantly in the vicinity of Madison, Wisconsin. Portions of the host tissue containing countless oospores were collected in February and placed in petri dishes. Three or four leaves were placed in each of eight petri dishes containing enough water to moisten the tissues well The water used was unfiltered lake water, In this condition part of the cultures were placed in the laboratory at ordinary room temperature and part out of doors, fully exposed to the weather. The latter part of March, the cultures were examined. In order to make a careful microscopic examination of the oospores, some of the infected material was killed, imbedded in paraffin, and stained. The fixing solution used was Flemming's medium. The material was cut 10 microns thick and stained with Flemming's triple stain, safranin, gentian-violet and orange G.

Observation and experiments. While examining the contents of one of the petri dishes several oospores were noticed that had hyaline sacs attached to them, in one of which the contents became a surging mass of moving globule-like bodies. These bodies were plainly zoospores. Later these escaped through a papilla or small opening, which was present on many of the sacs. This appearance at first suggested the idea that the oospores were germinating, but further observations showed that the phenomenon was instead the germination of some Chytrid parasitic within the oospores. Microtome sections were made of the infected oospores and stained as already explained. These showed delicate stalks extending from each zoosporangium into the oospore. Each of these stalks was much branched and formed a delicate system of rhizoids (fig. 3). Careful study has shown that it is a species of Rhizophidium probably *R. pollinis*, which has been reported by Schroeter (11) as infesting the oospores of *Sclerospora graminicola* and by Braun (3) in the pollen grains of *Pinus sylvestris*.

The sporangia are mostly pear-shaped or slightly oval and closely appressed to the surface of the oospore. They are often bunched on one side of the oospore and quite firmly attached so that the oospore can be rolled about in water without dislocating any of the sacs. Zoosporangia occur either singly or in clusters and the number on each oospore varies widely (figs. 1, 2). The size of these sacs varies remarkably, some are only 2 microns in diameter, while others are 40 microns (fig. 2). This variation in size is undoubtedly due, first, to the age, and second, to the number of separate infections occurring on the same oospore. In the young stages the sacs are filled with a translucent granular cytoplasm, but as they mature, the cytoplasm segregates into small dense masses with a highly refractive body at its center, doubtless an oil globule. While these changes are taking place in the cytoplasm of the sporangium, certain other changes are taking place in its walls. The outer wall of the sporagn-

ium is comparatively thick and colorless. It bulges out at several places. Soon the cell wall gelatinizes and an opening from 3 to 5 microns in diameter is formed, through which protrudes a cone-shaped portion of the contents of the sporangium. When cleavage is complete, the zoospores escape through this opening. Several swellings often develop on the same sporangium, but not all of them become openings so that the zoospores can escape. The pore is usually at the apical end (fig. 2a), though it is sometimes at the side of the sporangium. A few cases were found where the zoospores escaped through two pores at the same time though this was not the usual occurrence.

The successive stages in the growth and development of the sporangia were not critically studied, especially the early stages. It is known from the observations of Fisher (8) and Atkinson (1) that the zoospore develops a germ tube which penetrates the host. Favorable conditions and abundant material enabled me to study the germination of the zoospores. They put out a short germ tube which enters the oospore. The cell wall of the zoospore is left outside of the host and functions later as the sporangium. The germ tube later becomes the stalk of the sporangium. I have observed many small sacs, some only 5 microns in diameter, but have not found any as small as the zoospores. As a result nothing was learned regarding the entrance of the fungus into the oospore. According to earlier investigators, the old zoospore increases in size, after it has sent a germ tubeinto its host, until in some cases it becomes nearly as large as the infested oospore. The size of the sporangium at the time when the zoospores escape is not constant, but the size of the zoospores produced by the sporangia of various sizes are always very nearly the same. The time of the liberation of the zoospores is undoubtedly influenced by the amount of food supply available.

The escape of the swarm spores was carefully studied in at least twenty cases while hundreds of others were less critically observed, going through the same process. One by one they rush out through the opening of the zoosporangia and once outside they dart away with great speed. The time required for all the zoospores to escape from the sporangium varies. The shortest time observed was about ten minutes, while in some cases it required two or three hours before all of them had escaped. When the sporangium is nearly empty the zoospores are unable to escape so rapidly. They have considerable difficulty in finding the opening in the zoosporangium. This behaviour of the zoospores has been described by Atkinson (2) in another species of the genus Rhizophidium. The movement of the zoospores in the first sporangium studied began at 9.26 a.m. and at 9.35 a.m. another on the same oospore began to swarm, repeating the same phenomena which had already been observed on the first sporangium.

Six minutes later the third sporangium swarmed and four minutes later the fourth. At 10.04, the fifth sporangium also broke up into swarm spores. There were still other sporangia attached to the oospore in question, which swarmed at different times during the next ten hours, although longer period intervened between their successive swarming. In every case all of the zoospores succeeded in escaping.

The zoospores are very small, mostly spherical or pear-shaped, and about 3 microns in diameter. Near the center of each swarm spore is a highly refractive body, apparently a vacuole containing possibly an oily or fatty substance. The contents of this vacuole is so refractive that it is somewhat difficult to determine the outline of the zoospore without careful manipulation of the light and focusing of the microscope. As has been stated earlier the cytoplasm of the sporangia shows dense globules uniformly distributed through it. These are undoubtedly the early stages in the formation of the zoospore. At about this stage of development, nuclear division takes place which is followed directly by cleavage. The zoospores are uninucleate. A nucleus, 1 micron in diameter, having a distinct nucleole, can be made out when the zoospores are stained (fig. 5b). Attached at the anterior end of each zoospore is a single cilium which is from 9 to 12 microns in length (fig. 5a). It can readily be seen with the low power when the zoospore is quiet. The period of motility varies in different cases, the usual period being about thirty minutes. Upon losing its motility, the zoospore deposits a cellulose wall about itself and later pushes out a short germ tube. I was unable to follow the stages of penetration of the germ tubes into the oospores, although a large number of infection experiments were made, not only on the oospores of *Cystopus bliti*, but also on the oospores of other species belonging to the same family. The details of these infection experiments are given below. Many cases were noticed where the zoospores came to lie up against the oopsores, but the thick dark colored exospore made it impossible to follow the penetration of the germ tube produced by the zoospore. That it does not require a long period of time for infection to take place is plain from my infection experiments. As has been said before, the sporangia are attached to the oospores by a stalk which branches when it reaches the interior of the oospore forming a system of rhizoids. The stalk was originally the germ-tube of the zoospore. The rhizoids undoubtedly act as absorbing organs for the parasite. Sometimes the stalk connecting the zoosporangia with the system of rhizoids inside the infested oospore enlarges when it gets into the oospore. This condition much resembles the rhizoid development of certain species of Rhizidiomyces (fig. 4).

The contents of the oospores of Cystopus were devoid of nuclei and fat globules when several Rhizophidium sporangia had developed on them,

suggesting that this excess food supply had been taken up by the parasite. The cytoplasm of the infected oospores was not as dense as that of the noninfected spores (fig. 3).

Infection experiments. Oospores of *Cystopus bliti* were teased out of the host tissues and placed in distilled water in small watch glasses. These were exposed to infection by making transfers from the infected cultures. The oospores contained in four of the watch glasses were subject to infection, those in the two remaining were kept as controls. All of the cultures were kept in a moist chamber at room temperature. Daily examination was made by taking some of the contents from each of the cultures and examining it microscopically. On the fifth day, I was able to find in the cultures a few oospores that showed sporangia of *Rhizophidium pollinis*. The latter were small and occurred singly on the oospores. On the sixth day the number of oospores infected had materially increased and so had the size of the sporangia. On the eighth day after the four cultures were exposed to infection, about 1 per cent of the oospores in them were infected. The control cultures showed no infections at any time. The number of oospores that became infected gradually increased. Zoospores were found emerging from the zoosporangia on the ninth day after the oospores were exposed to infection. A large number of cultures were treated as described above, and infection resulted in from four to seven days. The sporangia swarmed in eight to eleven days after infection.

It was a very easy matter to maintain cultures of this Rhizophidium on the oospores of *Cystopus bliti* by infecting new cultures as the old ones died out. The chytrid was also kept growing on the oospores of Cystopus in one petri dish for one year by simply adding a new supply of oospores and water as needed.

It was also thought advisable to learn whether the oospores of other species of Cystopus were susceptible to infection by *Rhizophidium pollinis*. The oospores of *Cystopus candidus* and *C. cubicus* were exposed to infection by transferring infected oospores of *C. bliti*. Infection and sporangia development resulted in ten days as described for *Cystopus bliti*. On February 2, 1911, the oospores of *Peronospora effusa* and *Sclerospora graminicola* were also esposed to infection; here also infection resulted after six days. These results made it plain that *Rhizophidium pollinis* is quite cosmopolitan in its nature in that it grows readily on the oospores of three different genera of the Peronosporaceae. Four cultures consisting of a suspension of Rhizopus spores in water were made and exposed to infection February first. On February 8, small zoosporangia of Rhizophidium were observed on the Rhizopus spores. The two control cultures, however, continued to remain free from infection. I found the number of sporangia

developing on each Rhizopus spore was not nearly so great as previously reported for the oospores nor were the sporangia as large. This condition shows plainly that the number and size of sporangia are dependent, to a considerable extent at least, upon the amount of food material available from the host. No attempts were made to infect conidia of the Peronosporaceae, but it is very probable, in view of the results obtained with Rhizopus conidia, that they also can be infected.

Rhizophidium pollinis was found by Braun and described on the pollen grains of *Pinus sylvestris*. This fact led me to try to infect the pollen grains of the hyacinth and the calla-lily. Suspensions of pollen grains in water were made in eight watch glasses, four of each species of pollen. Two of each were exposed to infection by transferrring some of the infected oospores of *Cystopus bliti*. The experiment was started February first On the fifth, I observed small zoosporangia on the pollen grains of the hyacinth and the calla lily. The number of pollen grains that became infected was not great. The largest sporangia were only about one-fourth as large as the pollen grains. Seven days after the pollen grains had been exposed to infection, I saw sporangia swarming. It was noted that only the turgid and living pollen grains became infected. In no case did I find sporangia of Rhizophidium on pollen grains that had collapsed or disintegrated. The four control cultures, two of each species of pollen, were kept under observation for twenty days, remaining free from infection. This experiment makes it clear that the *Rhizophidium pollinis* parasitic on the oospores of *Cystopus bliti* may also become a parasite on pollen.

This *Rhizophidium pollinis* infesting the oospores of *Cystopus bliti* also produces a thick walled resting spore. These were not very numerous and developed only in my old cultures from one to two months after the sporangia had swarmed. The resting spore is produced inside the old sporangium. It has a thick, smooth, yellowish-brown wall and is filled with homogeneous cytoplasm containing several quite large fat globules. It is smaller than the average zoosporangium and more nearly spherical. The diameter of the resting spores varies from 20 to 32 microns. The number produced was so limited that no studies could be made of their development or germination.

It is well known, from the work of Braun, (3) Schroeter (11) Fischer, (8) Dangeard, (5) and Atkinson,(1) that resting spore production is a common occurrence in various species of Rhizophidium. But the species of Rhizophidium studied by the above investigators are predominatingly parasites on algae, hosts that are easily destroyed and low in food value. Oospores, on the other hand, are not easily destroyed and contain an abundance of food in proportion to the volume. This difference in the hosts attacked

may explain why resting spores are not produced in abundance on oospores of *Cystopus bliti*. It is, of course, possible that the temperature and moisture conditions of my cultures were not conducive to the best development of resting spores.

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PLATE IV. PHIZOPHIDIUM PARASITIC ON OOSPORES

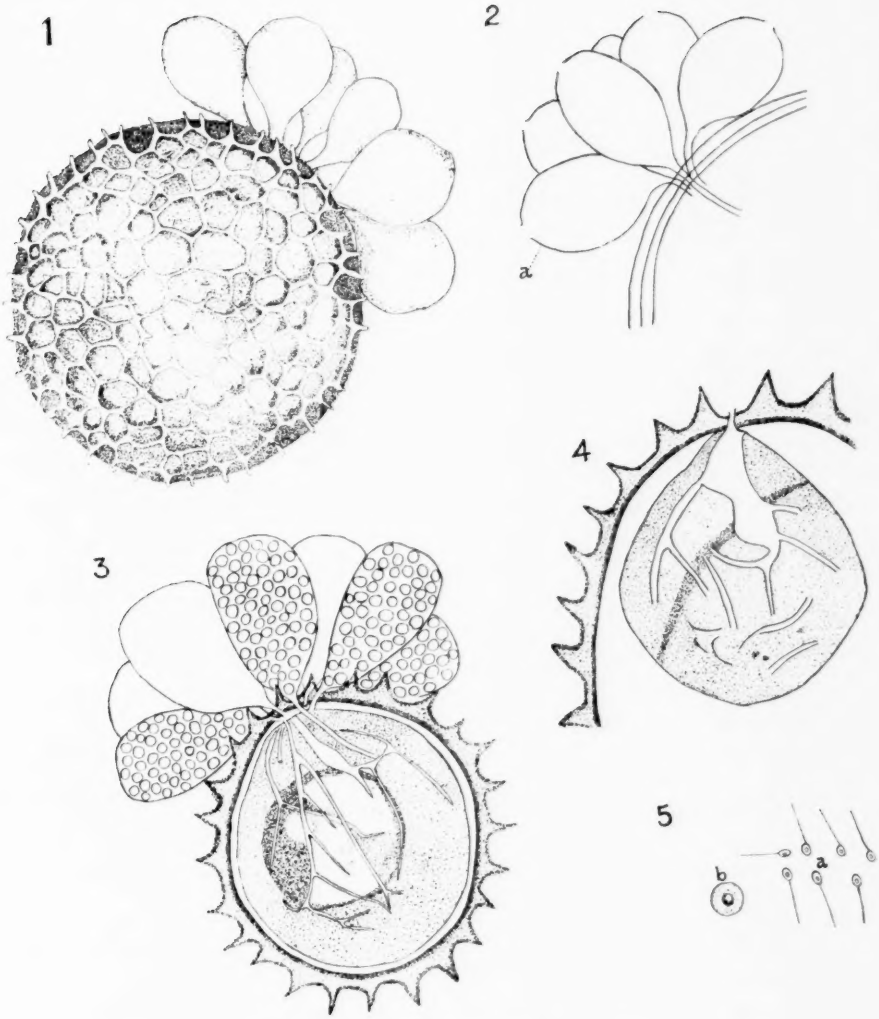
FIG. 1. An oospore of Cystopus bliti infested with Rhizophidium pollinis showing the sporangia of the chytrid.

FIG. 2. The empty sporangia of Rhizophidium.

FIG. 3. A section through an infected oospore showing the delicate rhizoids and stalks of Rhizophidium in the contents of an oospore. The sporangia are filled with zoospores.

FIG. 4. The rhizoids in the infected oospore sometimes are inflated.

FIG. 5. The zoospores of Rhizophidium pollinis as shown in *a* with cilia; *b*, a detailed drawing of a zoospore.



MELHUS: A SPECIES OF RHIZOPHIDIUM

NOTES ON DISEASES OF TREES IN THE SOUTHERN APPALACHIANS III

ARTHUR H. GRAVES

WITH PLATE V AND TEN FIGURES IN THE TEXT

The present paper deals with some observations on diseases of the spruce and hemlock, and will conclude the notes on coniferous species.

1. SOME DISEASES OF SPRUCE [PICEA ABIES (L.) KARST. AND P. RUBENS SARG.]

BLIGHT OF SEEDLINGS. *Ascochyta piniperda* Lindau, *Sclerotinia Fuckeliana* De Bary, and *Phoma* sp.

Four or five year old seedlings in a nursery at Biltmore, North Carolina, were found to be badly blighted, a trouble which was at first supposed to be due to the action of a late frost. Although the young trees may have been weakened through this agency, a more careful examination proved that this was not the real cause of the blight, a conclusion which was based on several considerations. First of all, the beds of seedlings were not blighted in a uniform manner, as would naturally have been the case had frost been the cause of the trouble. On the contrary, the disease appeared in spots. Again, the diseased shoots had apparently grown out too far before the blight set in to make the late frost, which in this neighborhood occurred on May 14 and 15, 1910, accountable for the trouble. Further, all the shoots on any given seedling were not blighted (pl. V. fig. 1), whereas frost usually kills all young shoots indiscriminately. Finally, fruiting bodies of fungi known to be of a parasitic nature were found on the blighted stems and leaves.

In many cases the blighted shoots were characterized by an abrupt downward curvature, appearing as if the fungi had so weakened the supporting tissues of the stem that they were no longer capable of holding it in its normal position. Such a shoot may be seen near the top of the seedling shown in figure 1, plate V. Or again, the shoot may have been attacked some distance from its base, so that merely the outer portion hung downward. Moreover, wherever the fungi had attacked the shoot it appeared much shrunken.

The field examination disclosed a luxuriant growth of some mycelium like that of *Sclerotinia Fuckeliana* on the diseased parts and this

fungus was tentatively noted as the cause of the trouble. Subsequent microscopical examination, however, revealed the presence of two other fungi, viz., *Ascochyta piniperda* and a species of Phoma, and at least one of these is parasitic. Besides these, a species of Macrosporium and one of Cladosporium were found, but they are believed to be purely saprophytic forms. The first three species are described below.

Ascochyta piniperda Lindau. This species, under the name *Septoria parasitica*, was first described in 1890 by Hartig[1] who had found it causing more or less damage in seed-beds of Norway spruce (*Picea Abies*), and also on the Sitka spruce, (*Picea sitchensis* Carr.) in Germany. Hartig demonstrated its parasitism by inoculation experiments. Later[2] he notes its occurrence on spruces which are thirty or more years old. The disease was again described by Rudolph[3] in 1898. In the same year Iwanoff[4] noted its presence on a plantation of one to four year old Norway spruce near St. Petersburg. Baudisch,[5] in 1903, stated that it was severe in Austria. Recently Rudolph[6] has investigated the trouble and finds four fungi present on the diseased shoots, which are, briefly, as follows: (1) the *Septoria parasitica* of Hartig, with the characteristic two-celled spores; (2) *Scleropycnis abietina* Sydow, with one-celled flask-shaped spores; (3) a Phoma with more or less spherical spores which vary considerably in their dimensions; (4) a form which resembles *Cytispora Abietis*. Inoculation experiments were carried out with the first three forms, but without result.

Since Saccardo[7] and Lindau[8] have defined the genus Septoria as typically containing leaf-inhabiting forms, it seems best in the case of this fungus, which attacks the stem, and also has the shorter spores, characteristic of Ascochyta, to adopt Lindau's[9] name, *Ascochyta piniperda*. A change in the specific name was necessary on account of the fact that an *Ascochyta parasitica* already existed.

[1] Hartig, R., Eine Krankheit der Fichtentriebe. Zeitschr. f. Forst u. Jagdw. **22**: 667–670. f. 1–2. 1890.

[2] Hartig, R., Septoria parasitica m. in älteren Fichten beständen. Forstl. naturw. Zeitschr. **2**: 357–359. 1893.

[3] Rudolph, Vortrag über die Pilzkrankheit Septoria parasitica. Forstl. naturw. Zeitschr. **7**: 265–273. 1898.

[4] Iwanoff, K. S., Die im Sommer 1898 bei Petersburg beobachteten Krankheiten. Zeitschr. f. Pflanzenkr. **10**: 100. 1900.

[5] Baudisch, F., Notizen über Septoria parasitica R. H., Fusoma Pini R. H. and Allescheria Laricis R. H. Centralbl. f. das gesamte Forstw. **29**: 461. 1903.

[6] Rudolph, Beiträge zur Kenntniss der sogenannten Septoria-Krankheit der Fichte. Naturw. Zeitschr. f. Land u. Forstw. **10**: 411–415. 1912.

[7] Saccardo, P. A., Sylloge Fungorum **3**: 474. 1884.

[8] Lindau, G., In Engler und Prantl, Die natürlichen Pflanzenfamilien **1**: 378. 1900.

[9] Lindau, G., Loc. cit., p. 367.

In our specimens the pycnidia are more or less imbedded in the tissue of the stem (text fig. 1), usually broadly oval in shape and averaging 85μ in diameter, although specimens much smaller than this occur. Hartig[10] found them to contain one or more cavities, but our specimens never showed more than one. Hartig gave no measurements of the pycnidia, but his figures show little discrepancy here, if any. His pycnidia, however, seem to project out from the host farther than ours. The pycnidia contain an abundance of two-celled, ellipsoid, hyaline spores, averaging 9 x 1.5μ (text fig. 2). Hartig gave only the length of the spores, 13 to 15μ, which is a little long for our specimens. However, our spores may not be mature, or it is possible that we have another species or variety.

To the writer's knowledge this fungus has not been before reported in America. If the form described above is the same as that of Hartig and other European observers, its presence here is of considerable importance, when we recall the large amount of damage it has caused in Europe, not only in seed-beds, but also in the case of older trees. Its characteristics should be borne in mind, and its occurrence watched for.

Sclerotinia Fuckeliana De Bary. As was stated above, the vigorous mycelium of this fungus was so conspicuous that it was taken in the field to be the sole cause of the blight. Its parasitism on the Douglas spruce (*Pseudotsuga taxifolia* (Lam.) Britton) was established in 1888 by Tubeuf,[11] who successfully performed inoculation experiments and described the form as *Botrytis Douglasii*. From infection experiments by Behrens[12] and Tuzson[13] it would seem, however, that the fungus is the same as *Botrytis cinerea* Pers., and Smith[14] so considers it, while Istvanffi[15] has recently demonstrated that *Botrytis cinerea* has *Sclerotinia Fuckeliana* as its perfect stage.

While *Botrytis Douglasii* has frequently been reported in Europe as causing a disease of conifers, the occurrence of the fungus on coniferous hosts in this country is limited, if one were to judge from the literature. As far as the writer can ascertain, the only reference of this sort is in a recent

[10] Hartig, R., Eine Krankheit der Fichtentriebe. Zeitschr. f. Forst u. Jagdw. **22**: 669. 1890.

[11] Tubeuf, C. von., Beiträge zur Kenntniss der Baumkrankheiten. II. Botrytis Douglasii, pp. 4–8. Berlin. 1888.

[12] Behrens, J., Phytopathologische Notizen. I. Botrytis Douglasii Tub. Zeitschr. f. Pflanzenkr. **5**: 136–141. 1895.

[13] Tuzson, J., Ueber die Botrytis-Krankheit junger Nadelholzpflanzen. (Botrytis cinerea Pers.) Zeitschr. f. Pflanzenkr. **11**: 95–98. 1901.

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[15] Istvanffi, G. De., Études microbiologiques et mycologiques sur le rot gris de la vigne. Ann. inst. cent. ampel. roy. Hongrois. **1905–6**: 183–360. (Abstr. in Expt. Sta. Rec. **17**: 985–986.)

note by Weir[16], who describes a form, apparently the same as *Botrytis Douglasii*, on young shoots of Douglas fir (*Pseudotsuga taxifolia*), *Abies grandis*, *Tsuga heterophylla*, and *Larix occidentalis* in the Priest River Valley, Idaho. In this connection it should be added that several years ago a disease was observed on young seedlings of *Sequoia sempervirens* and *S. Washingtoniana* in the Yale Forest School nursery, the causal fungus of which was determined as this species, and its parasitism proved by inoculation. The writer suspects that the trouble is of fairly common occurrence in this country, on coniferous stock.

Phoma sp. On the ends of some of the withered shoots appeared sparingly the fruiting bodies of a species of Phoma containing very minute spores. The pycnidia were more or less globular, having their origin beneath the epidermis, and varied from 125 to 170μ in diameter (text fig. 3).

FIG. 1. Section through pycnidium of Ascochyta piniperda Lindau on Picea Abies. × 350.

FIG. 2. Spores of the same. × 350

FIG. 3. Pycnidium of Phoma sp. on Picea Abies. × 60

FIG. 4. Spores of same. × 500

The spores, averaging 3 x 0.75μ, were very numerous, hyaline, ellipsoid, but often with a construction in the middle, where there sometimes, in a certain light, appeared to be a septum (text fig. 4). Just what species this is, it is difficult to judge, as it agrees fairly well with the descriptions of several, e.g., *Ph. Fuckelii* Sacc. and *Ph. minutula* Sacc., but neither of these seems to grow on conifers. However, it does not seem to be the *Phoma piceina* recently described by Peck,[17] and reported by Spaulding[18] as being destructive to the Norway spruce.

[16] Weir, J. R., A Botrytis on conifers in the northwest. Phytopathology **2**: 215. 1912.

[17] Peck, C. H., N. Y. State Museum Bul. 150: 35. 1911.

[18] Spaulding, P., Notes upon tree diseases in the eastern states. Mycologia **4**: 148-151. 1912.

TWIG BLIGHT

Vigorous young trees of Norway spruce, 6 to 15 feet high, planted out in the Biltmore Forest several years ago, near a brook, showed the young shoots of the year in many cases badly diseased, having been arrested in a rather early stage of development, with their leaves not yet matured, still bunched up together, and the whole shoot drooping. Other shoots which had simply their tips diseased had retained a more erect position, but the stem here was considerably shrunken.

As in the case of the blight of young seedlings described above, the symptoms all simulated those of frost injury; but here, again, the same contradictory evidence presented itself, first of all in the fact that many of the shoots were entirely uninjured. On the other hand, no fungi were found which have been definitely proven to be parasitic. Nevertheless, two species occurred which deserve notice.

Pestalozzia sp.. On the leaves of some of the withered shoots the acervuli of a species of Pestalozzia were found sparingly. At first this was supposed to be *P. funerea* Desm. which has been reported by Böhm,[19] Rostrup,[20] Ritzema-Bos,[21] Smith,[22] Fisher,[23] and others as causing more or less damage to conifers. Although Böhm conducted inoculation experiments with this species with a view to a decision of the question of its parasitism, they were not very successful, and the matter seems to have been left more or less in doubt ever since.

Spaulding[24] has also noted a species of Pestalozzia, which, according to Hartley,[25] is *P. funerea*, as being very destructive to seedlings of *Pinus divaricata* (Ait.) Du Mout de Cours. and *Pinus ponderosa* Laws., and has proved its parasitism by inoculation. Hartley's inoculation experiments with this species on arborvitae (*Thuja occidentalis*) have given negative results.

[19] Böhm, B., Ueber das Absterben von Thuja Menziesii Dougl. und Pseudotsuga Douglasii Carr. Zeitschr. f. Forst u. Jagdw. **26**: 63–71. f. 7. 1894.

[20] Rostrup, E., Mykologiske Meddelelser. (V) Spredte Jakttagelser fra 1893. Saertryk of Botanisk Tidkrift 19[3]: 1895. 18 p. (Abstr. in Just's Bot. Jahresber. **23**[1]: 368–369. 1895.)

[21] Ritzema-Bos, J., Ueber Einschnürungen an den Stämmchen junger Douglastannen, verursacht durch Pestalozzia Hartigii Tub. Tijdschr. over Plantenziekten **4**: 161. 1898. (Abstr. in Just's Bot. Jahresber. **27**[2]: 432–433. 1902.)

[22] Smith, W. G., A disease of ornamental conifers. Gard. Chron., 3d Ser. **26**: 354. 1899.

[23] Fisher, J., Two fungous diseases of coniferous trees. Agr. Jour. Union So. Africa **3**: 389–391. 1912.

[24] Spaulding, P., A blight disease of young conifers. Science N. S. **26**: 220–221. 1907.

[25] Hartley, C., The blights of coniferous nursery stock. U. S. Dept. Agr. Bul. 44. 1913.

In general, our form agrees well with Saccardo's[26] description of *P. funerea* Desm. The spores are usually five-celled, with the three central cells darker colored, and the end cells hyaline, although sometimes four-celled with the two central cells darker. Three or four beaks are present, while the spore is borne on a comparatively short stipe. Exclusive of the terminal beak and the stipe, the spores average 25 x 5 μ. The beaks average 11μ and the stipe 5μ in length (text fig. 5).

In one particular, however, the spores differ from those of *P. funerea*, namely, in having only one terminal beak produced from the tip of the end cell, while two or three lateral ones originate from the base of this cell. Such a feature is not mentioned in Saccardo's description of *P. funerea*, nor do we find it figured for this species anywhere in the literature. Moreover, exsiccati examined, (De Thümen, Myc. Univ. 884, 1877; N. A. F. 2389, 1890; Fungi Col. 3535, 1911) show the beaks of *P. funerea* all produced from the tip of the end cell.

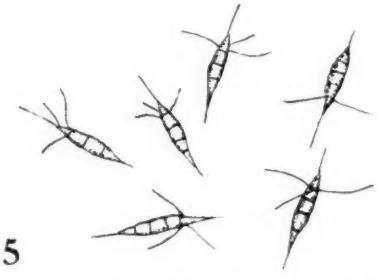


FIG. 5. Spores of Pestalozzia sp. on Picea Abies. × 350
FIG. 6. Spores of Phoma piceana Karst. on Picea Abies. × 500

Morphologically, our form is perhaps a variation from the type, but pathologically its affects appear to be similar to those of *P. funerea* as noted by the observers mentioned above.

Phoma piceana Karst. A species of Phoma was found on the withered dead leaves which corresponded with the description of *Phoma piceana* Karst.[27] while the pycnidia averaged 300μ in diameter, as against 500 to 800μ of Karsten, the hyaline spores measured 5 x 1μ and were slightly curved, these latter points coinciding exactly with Karsten's description (text fig. 6). This species is probably saprophytic, or at least of secondary nature.

[26] Saccardo, P. A., Sylloge Fungorum **3**: 791. 1884.
[27] Karsten, P. A., Fragmenta mycologica. VIII. Hedwigia **23**: 18. 1884.

HEART ROT. *Trametes Pini* (Brot.) Fr.

The sporophores of the fungus causing this disease were collected from the trunk and limbs of a living tree of *Picea rubens* Sarg. near Jones' Knob in Haywood County, North Carolina, at an elevation of about 6000 feet.

FROST INJURY

Near Jones' Knob, North Carolina, at an elevation of about 6000 feet, this year's shoots of some otherwise healthy red spruces (*Picea rubens*) were withered, and hung downward, having apparently been arrested in an early stage of development, in some cases even the bud scales remaining at the tip of the shoot (pl. V, fig. 5). Examination showed a septate, colorless mycelium overrunning these shoots, but no spores had been developed. Taking into consideration the uniform way in which the shoots were thus injured, their early stage of development, and the high altitude, it is probable that in this case frost was the primary cause of the trouble.

2. SOME DISEASES OF HEMLOCK [TSUGA CANADENSIS (L.) CARR. AND T. CAROLINIANA ENGELM.]

TIMBER ROT. *Fomes pinicola* Fr.

The fruiting bodies of the causal fungus of this disease were collected on dead hemlock (*T. canadensis*), both fallen and standing trees, in the extensive forests in Transylvania and Jackson Counties, North Carolina. Although the fungus was not observed on living trees, it is well known to attack them, gaining an entrance through wounds or dead branch stubs. After the tree dies, the fungus continues to live in the trunk, ultimately rendering it entirely worthless (Von Schrenk.[28])

The species occurs abundantly in temperate North America and in Europe, and is of especial interest botanically in that it is not entirely confined to coniferous hosts, as are, for example, *Trametes Pini* and *Polyporus Schweinitzii*. Murrill,[29] Romell,[30] Atkinson,[31] Pennington[32] and others

[28] Von Schrenk, H., Some diseases of New England conifers. U. S. Dept. Agr., Div. of Veg. Phys. and Path. Bul. 25: 24–31. 1900.

[29] Murrill, W. A., The Polyporaceae of North America. III. The genus Fomes. Bul. Torr. Bot. Club. **30**: 228. 1903.

[30] Romell, L., Some fungi growing on both coniferous and deciduous trees. Mycologia **1**: 265–267. 1909.

[31] Atkinson, G. F., Studies of some shade tree and timber-destroying fungi. Cornell Univ. Agr. Expt. Sta. Bul. 193: 227. 1901.

[32] Pennington, L. H., Fomes pinicola and its host plants. Mich. Acad. Sci. Rpt. **9**: 80–82. 1907.

have reported it on broad-leaved trees, such as the sugar maple, beech, yellow and white birch, and alder.

From the standpoint of the forester it is a dangerous enemy, not only because of this omnivorous habit, but chiefly on account of its ability to attack living as well as dead trees. It can thus continue to flourish as a saprophyte in the trunk after the tree is killed, and, by the uninterrupted production of sporophores, spread the disease to other individuals.

LEAF RUST. *Pucciniastrum Myrtilli* (Schum.) Arth.

As already noted by Spaulding,[33] the writer found this rust on trees of *Tsuga canadensis* and *T. caroliniana* in North Carolina. The collections were made at Biltmore, July 19 and 24, 1910. At the time, the alternate host was unknown, but according to recent investigations by Clinton[34] and Fraser,[35, 36] it is a member of the Ericaceae.

The disease does little damage to the trees, not being even apparent until the leaves are closely examined. Then certain of them may be seen to be of a yellowish cast, and examination reveals the aecia of the fungus on the under side of the leaf. Not all the leaves on any given branch are affected —only a few, here and there—and in this manner they may be scattered over the whole tree.

TWIG BLIGHT. *Rosellinia* sp.

This disease was observed on two large hemlocks (*Tsuga canadensis*) a short distance apart in the Pisgah Forest, Transylvania County, and again on a single tree of the same species near Plott's Balsam, in Haywood County, North Carolina. Although it was found only in these two localities, nothing can be deduced from this as regards its prevalence in the region, as no especial attention was paid to the hemlocks in the general survey of tree diseases. Where the disease was found, however, it seemed to cause little damage.

The most striking symptom was a yellowing of the leaves, particularly on lower branches of otherwise healthy trees, but in addition to this, a closer examination showed that many of the leaves had already fallen off. Others, in many cases, were fastened to each other or to the branch by a growth of grayish or yellowish-brown mycelium which invested more or

[33] Spaulding, P., The rusts of Tsuga canadensis. Phytopathology **1**: 94–96. 1911.

[34] Clinton, G. P., Notes on plant diseases of Connecticut. Conn. Agr. Exp. Sta. Rpt. **1909–1910**: 719. 1911.

[35] Fraser, W. P., Cultures of heteroecious rusts. Mycologia **4**: 175–193. 1912.

[36] Fraser, W. P., Further cultures of heteroecious rusts. Mycologia **5**: 233–239. 1913.

less of the branch and the short leaf petioles, sometimes including also a considerable portion of the base of the leaf. This mycelium was not at all conspicuous, but where most vigorous could be seen by careful examination with the naked eye, or readily with the hand lens (text figs. 7 and 8).

Where the mycelium was thickest, small, globular, or often somewhat flattened or dome-shaped, fruiting bodies about ½ mm. in diameter appeared, now in small groups and now occurring singly. In color they were black or often gray, the latter hue being caused by a fine investment of mycelium. They were entirely free from the tissue of the stem, but well enveloped about their basal part by the mycelium. However, they were not sunken in the mycelium to the extent, for example, of those of *Herpotrichia nigra* Hartig and *Neopeckia Coulteri* (Pk.) Sacc. described in a recent paper by Sturgis.[37]

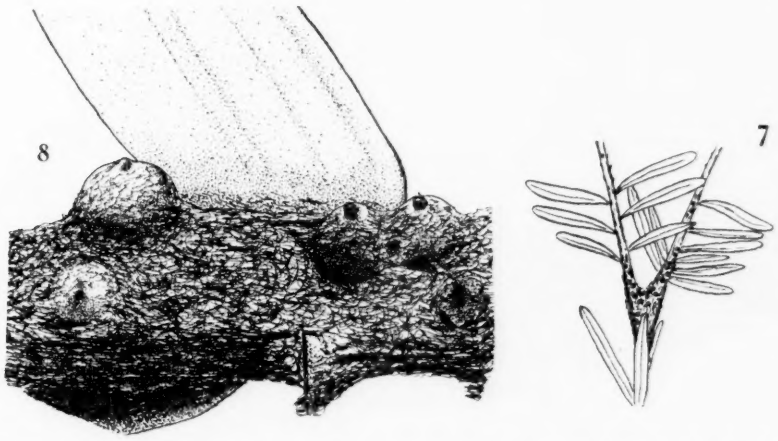


FIG. 7. Portion of diseased branch of hemlock, Tsuga canadensis, showing mycelium and fruiting bodies of Rosellinia sp. Natural size.

FIG. 8. The same. × 17

(Pl. V, figs. 2, 3, 4 and 6). Although it may be seen that some of the fruiting bodies are considerably depressed above (pl. V, fig. 3), this is probably not a normal condition, but is due, perhaps, to drying out. Plate V, figure 6 and text figure 8 show what is believed to be the normal type, with a distinct papillate opening at the apex.

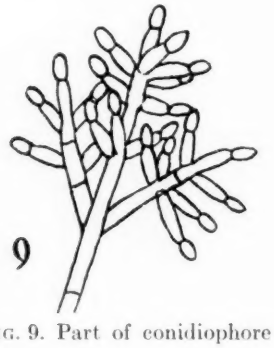
Although a great many of the fruiting bodies were examined, all were either immature or sterile, containing neither asci or spores, so that determination of the species or even the genus was exceedingly difficult. Some of the material was sent to Dr. Farlow, however, who kindly identified

[37] Sturgis, W. C., Herpotrichia and Neopeckia on conifers. Phytopathology **3**: 152–158, pls. 2. 1913.

the fungus as belonging to the genus Rosellinia, the general habit being that of the group to which *R. subiculata* (Schw.) Sacc. belongs, although it is probably not that species.

As is evidently a fairly common occurrence in this genus,[38] the mycelium at many points bore conidia in abundance, so that, viewed with a hand lens, it exhibited the general powdery appearance of a Botrytis in fruit. Microscopical examination revealed copiously branched conidiophores, the branches very often in whorls (text fig. 9), and each bearing at its apex an oval, transparent spore measuring 6.5 x 4μ (text fig. 10). Whether or not the branches bore spores along their sides could not be determined with certainty, but this did not seem to be the case. The mycelium varied in width from 2.5 to 5μ.

If one were to judge merely from its appearance on living trees, on branches which seemed healthy up to the point of the location of the mycelium,



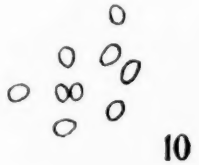


FIG. 9. Part of conidiophore with spores, borne on mycelium of Rosellinia sp. × 496

FIG. 10. Spores of same, showing variation in size. × 500

the fungus looked parasitic, but whether it is really such, of course only inoculation experiments can prove. It should also be noted in this connection that the diseased branches appeared too numerous and not always in a proper position for the trouble to be interpreted as due primarily to suppression. Although diseases have been described as being caused by parasitic species of Rosellinia, the only species thus far known to attack living trees is *R. quercina* Hartig, which lives in the roots of oak seedlings, causing their leaves to become pale and to dry up.

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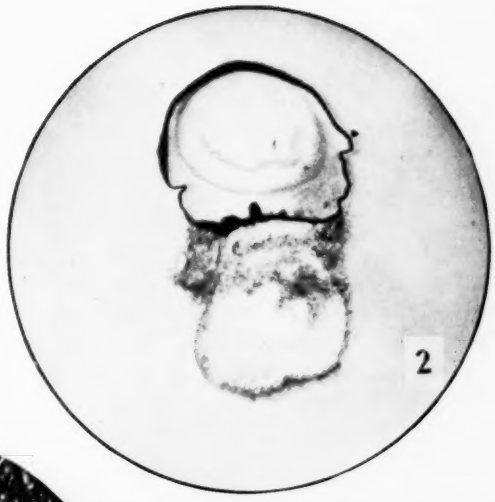
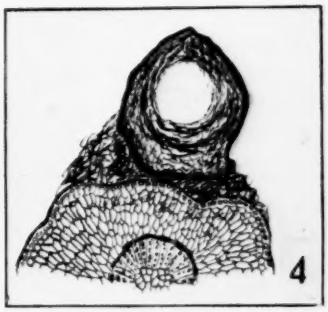
[38] Winter, G., In Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz 1^2: 220 ff. 1887.





GRAVES: DISEASES OF TREES

Explanation of Plate V

Fig. 1. Part of diseased seedling of Picea Abies, showing blighted shoots. × $\frac{2}{3}$.

Fig. 2. Photomicrograph of section through fruiting body of Rosellinia sp., on Tsuga canadensis branch. × 50.

Fig. 3. Photomicrograph of part of diseased branch of Tsuga canadensis, showing mycelium and fruiting bodies of Rosellinia sp. × 10.

Fig. 4. Drawing of section through fruiting body of Rosellinia sp., on Tsuga canadensis branch. × 35.

Fig. 5. Portion of branch of Picea rubens, injured by frost. × $\frac{1}{2}$.

Fig. 6. Photomicrograph of part of diseased branch of Tsuga canadensis, showing mycelium and fruiting bodies of Rosellinia sp. × 10

THE DAMPING-OFF OF CONIFEROUS SEEDLINGS

PERLEY SPAULDING

WITH PLATE VI AND ONE FIGURE IN THE TEXT

The raising of coniferous seedlings has of late years assumed a degree of importance in this country that is not generally realized by the public. This is caused by the use of such seedlings for the purpose of reforesting large tracts of barren or waste lands. A number of the States are doing extensive work of this kind, and private individuals and corporations are using a rapidly increasing number of seedlings for ornamental and commercial plantings. Besides all this, the Forest Service is planting out great numbers in the various reforesting projects which it is carrying on in different parts of the country.

One of the most serious enemies with which the nurseryman has to contend is the disease known as damping-off. It is a universal trouble in seed beds of nearly all kinds. It is aggravated by adverse weather conditions and is especially difficult to stop when once started in a coniferous seed bed, owing to the close proximity of the plants to each other and because unfavorable weather conditions can not be overcome in the open air. The disease usually appears soon after the tiny plantlets have pushed their way through the soil, and it continues for some weeks, during which time the plants are forming their woody stems. Occasionally it kills the seedlings before they appear above ground. Unless the weather is very favorable for the growth of the fungus the trouble stops when the stem begins to acquire its woody cylinder, but it does not always do so. During this brief period damping-off may have destroyed all or nearly all of the seedlings in an infected seed bed or nursery. The damage caused by the disease may be complete, as it is usually too late to make a second sowing that season, and the necessary quantity of young stock for planting out the next spring, or later, may be very greatly reduced.

In the early work of attempting the control of damping-off of tree seedlings the main object in view was that of controlling outbreaks of the disease by treatment of the germinated seedlings after the disease had made its appearance. The difficulties in discovering a substance which was effective and which did not injure the seedlings led to the giving up of this idea and the substitution of soil treatment before sowing the seed. The former idea has been pretty thoroughly tested, but the indications were not con-

sidered promising enough to warrant further work. The latter idea has been tested in a fragmentary way, and the results seem to justify further investigation. In the following pages it must be remembered by the reader that this account applies only to the specific localities mentioned, all of which are in the eastern region of plentiful precipitation.

SYMPTOMS OF THE DISEASE

While, for convenience, the trouble is spoken of here as a single disease, it is really a series of diseases caused by a number of distinct types of fungi. As stated below, an indefinite number of species of Fusarium are capable of causing this disease, and the statements in this paper apply especially to the trouble caused by them. But Pythium has been found causing the disease, and Rhizoctonia has been found in connection with the trouble, occurring as if a parasite.

The disease usually begins at or just below the surface of the soil where there is plenty of moisture and air. Occasionally it begins lower down on the roots, but this is apparently due to abnormal conditions. While damping-off may kill the germinating seedlings before they push through the soil, it usually attacks them a few days after the seedlings have come up. At this time a few seedlings are found which have fallen over and which still may have the upper portion green and fairly turgid. The disease first appears as a small, watery spot on the stem. A little later the stem becomes a trifle shrunken at this spot, soon bends, and the plant falls over. The fallen plant loses its green color, dies, withers, and finally disappears entirely. The disease spreads rapidly around the plants first affected until more or less extensive areas are denuded of seedlings. If the seedlings are small, like those of *Pinus sylvestris* Linn., the fungus rarely becomes evident on the exterior of the diseased plants. On the other hand, if they are large, like those of *Pinus ponderosa* Laws., the mycelium and fruiting bodies of the fungus may often be found on the exterior of the dead seedlings. The attacks ordinarily are of brief duration, the seedlings commonly being susceptible for only a few weeks after germination. But the too frequently empty seed rows and beds testify eloquently to the destructive power of this disease during the brief period in which it makes its attacks.

ORGANISMS CAUSING THE DISEASE

In Europe there are several fungi which are known to cause the damping-off of tree seedlings. *Pyhtophthora omnivora* de Bary is widely distributed and well known by reason of its attacks upon seedlings both of deciduous and coniferous trees (1, 2, 4, 5, 6, 7, 8, 10, 31, 32, 40). *Botrytis douglasii* Tubeuf is known to cause a serious disease of coniferous seed-

lings (9, 24). Still another fungus, about which little is known, and which causes a very serious local damping-off of coniferous seedlings, is known as *Fusoma parasiticum* Tubeuf. (12, 17, 23, 36, 26, 31, 40) This has been found in Baden and Bavaria in Germany, in Scandinavia and in England.

In the earlier work herein described it was found that Botrytis practically never attacked young coniferous seedlings as an active parasite, but did often occur as a secondary organism on damped-off seedlings. In most cases various forms of Fusarium were apparently the real parasitic agents. These were often secured in pure cultures directly from the diseased seedlings. Moreover, greenhouse tests showed that all of 44 different strains and species of Fusarium obtained in pure cultures were capable of attacking young pine seedlings. The Fusarium cultures were obtained from the sources and localities indicated below. Where no locality is mentioned they were from St. Louis, Missouri, and vicinity. The plants from which the cultures were obtained are as follows: Agave, leaf; almond seedling, stem; asparagus, stem; cauliflower, head; Concord grape, fruit; corn, kernels (Lincoln, Nebraska);[1] corn, kernels, (Bethel, Vermont); cotton;[2] dahlia, root;[3] dewberry, root gall;[3] Euphorbia, leaf;[3] Ficus, root gall;[3] gourd, fruit;[3] grape, stem; Johnson grass, stem;[3] lily, stem; moss rose, fruits;[3] oak, leaf (Avalon, California);[3] onion, bulb; orchid, leaf; peach, fruit; peach, root gall;[3] *Pinus sylvestris*, seedlings; *Pinus divaricata*, seedlings, (Halsey, Nebraska); *Pinus strobus*, seedlings (Saranac Inn, New York); *Pseudotsuga mucronata*, seedlings (Halsey, Nebraska and Saranac Inn, New York); *Podophyllum*, leaves; potato, tuber; pumpkin, fruit; paper pulp, (Glens Falls, New York); red raspberry, fruit[3] and canes; black raspberry, cane; rose, fruits;[3] sawdust; squash, fruit; sugar beet, root (Ames, Nebraska); sweet pea, stem (Mountain View, California);[3] sweet potato; tobacco, seedlings (Madison, Wisconsin); tomato, fruit; white pine, wood (Ashland, Wisconsin); and watermelon.[2] Pure cultures of Sclerotinia from cabbage,[3] and of *Thielavia basicola* Zopf. from tobacco seedlings[4] were tested in the same way at the same time, but with negative results.

The test was as follows. Pure cultures of the fungi were grown on boiled rice in tubes (not too wet), and 6-inch pots of garden soil were steam sterilized. Seeds of *Pinus ponderosa* were soaked for several minutes in a $33\frac{1}{3}$ per cent solution of commercial sulphuric acid, then for an equal time in a 10 per cent solution of sodium hydrate, and finally were washed with distilled water. The seeds were then scattered in equal quantities (by

[1] Pure culture of Fusarium moniliforme Sheldon supplied by Dr. John L. Sheldon.

[2] Pure cultures of wilt organism, Fusarium vasinfectum, supplied by Dr. Erwin F. Smith.

[3] Pure culture supplied by Dr. G. G. Hedgcock.

[4] Pure culture supplied by Mr. W. W. Gilbert.

bulk) in the sterilized pots of soil. A culture tube of rice thoroughly permeated with the vigorously growing mycelium of a Fusarium was scattered with the seed in each pot. The whole was then covered with steam-sterilized soil and a glass plate laid over each pot. In the same way each strain of Fusarium was placed in a pot and all were tested simultaneously. The watering was entirely from below, that is, by pouring into the saucer in which the pot sat. Six checks were used. In 28 out of the 44 forms of Fusarium tested, aggressive attacks were made on the seedlings, while the remainder were weakly parasitic. There can be no doubt that the conditions under which the test was conducted greatly favored the attack of the fungi. Nevertheless, the experiment is of value in showing the possible parasitism of the species of Fusarium thus tested when the conditions are favorable (see text figs. 1 and 2).

Fig. 1

Fig. 2

FIG. 1. Fusarium from diseased seedling of Pinus strobus.
FIG. 2. Fusarium moniliforme from corn kernels.

Because of the extremely unsatisfactory state of our knowledge of the entire genus Fusarium, no attempt was made to identify the different forms used in this test. A small group, which, like *F. moniliforme* Sheldon, has simple microconidia borne in chains, were especially virulent in their attacks (fig. 2). At the conclusion of the experiment the form of fungus originally used was recovered from the diseased seedlings in every case tested. Two checks became contaminated toward the end of the experiment, but they all remained healthy about two weeks, so this is not considered to have any important bearing on the results. Eight of the cultures used were secured from damped-off tree seedlings in nurseries. These did not prove to be especially virulent parasites, but were about medium in this respect (see fig. 1).

In the earlier work the writer looked for *Pythium debaryanum* Hesse (3, 11, 15, 16, 30) as a parasite, but without success. As a result of the work of Hartley in 1909 on damping-off (39, 43, 44), the writer learned of the presence of *Pythium debaryanum* in western nurseries as the cause of damping-off in coniferous seedlings that year. In an eastern nursery in the spring of 1910 the writer found this fungus causing disastrous damping-off of recently-germinated seedlings of *Picea engelmanni* Engelm., *P. parryana* (André) Parry, *Tilia europaea* L. and *T. ulmifolia* Scop., the fungus being isolated in pure cultures directly from each host. *Rhizoctonia* has been found by the writer, but its parasitism was uncertain, as no tests were made to find this out. *Phytophthora omnivora* has not been found by the writer.

TESTS OF FUNGICIDES

Early in the spring of 1904 the damping-off of some recently-germinated seedlings of *Pinus ponderosa* which were raised in the greenhouse for experimental use directed the writer's attention to this disease. A considerable number of greenhouse experiments were carried out at various times up to the summer of 1907. These indicated that formalin, copper sulphate, and mercuric-chloride solutions were efficient soil sterilizers when the soil was in pots, flats, or greenhouse benches; but the two last distinctly affected the seedlings, and it was felt that the experiments should be carried on in the open air and under actual nursery conditions in order to be of practical value to nurserymen. In 1906 experiments on a small scale at Saranac Inn, New York, gave encouragement to continue the tests another season, although little damping-off occurred that year.

Experiments were carried on in 1907 at the same place, with sulphuric acid (13, 14, 18, 22, 25, 27, 28, 35, 38, 43, 44), formalin (19, 20, 21, 29, 41, 33, 34, 35, 42), sulphur, copper sulphate and lime powdered and mixed at the rate of 1 pound of the sulphate to 8 pounds of the powdered lime, potassium permanganate, and potassium sulphide. Owing to the absence of damping-off in the experimental beds no definite results were obtained, except with formalin, with which the results were adverse. Formalin was applied on an 8 by 4-foot plat at the rate of 4 fluid ounces to $2\frac{1}{2}$ gallons of water on May 25 before sowing the seed. On June 20, after the seedlings had germinated, a second application of 2 gallons of water and 1 ounce of formalin was made. At one end, 4 by 4 feet in size, the copper sulphate and lime powder was later applied. On June 21 damping-off began. With formalin alone about 50 per cent of the total stand was lost before the attack ceased; using formalin followed by copper sulphate and lime powder about 35 per cent was lost; while in the dry check plat, which was left entirely untreated, none were lost. Counts made a month later showed

that the plat treated with formalin alone had 175 plants per square foot, while the check plat had 350 per square foot. Notes taken a year later showed that the stand of the check plat was still far thicker than that of the formalin plat.

The copper sulphate and lime powder was used very extensively in the entire nursery in 1907 to stop an attack of damping-off. No checks were left, so the stopping of the attack can not be wholly ascribed to the treatment. Everything seemed to indicate that it was of some use, however.

At Burlington, Vermont,[5] in 1907, a number of tests of the above-named chemicals were made, but only the following were significant. A bed, 4 by 12 feet which had just entirely damped-off was replanted with seed of *Pinus sylvestris*. Before sowing the seed the bed was divided into thirds. The west one-third was left as a check and was untreated, and the remaining two-thirds was divided lengthwise into two equal plats. One was treated with about 10 quarts of 1 per cent solution of formalin in water, the other with an equal amount of 0.5 per cent solution. The bed was then covered with canvas and left for 72 hours, after which it was uncovered and left open two days. The seed was then sown as usual.

The formalin affected the germination, so that the stands were as follows:

Check	3200 plants
Weak formalin	2400 plants
Strong formalin	1700 plants

The damping-off set in shortly after and left the stand as shown below (pl. V, fig. 3).

Check	lost	90 per cent	320 left
Weak	lost	9 per cent	2190 left
Strong formalin	lost	7½ per cent	1570 left

A bed of *Pinus sylvestris* which had started to damp-off was divided into equal plats of 4 by 4 feet. One was treated with a solution of 1¼ ounces sulphuric acid to 3 gallons of water, another was treated with a solution of 12 grams of potassium permanganate in 3 gallons of water, while the middle plat was wet with the same amount of water and left for a check. Counts shortly after showed the following stands:

Check	1050 damped-off	220 healthy.
Potassium permanganate	1070 damped-off	330 healthy.
Sulphuric acid	675 damped-off	490 healthy.

[5] See Jones (34) for an account of this experiment, which was carried on entirely under his direction. Dr. Jones also aided very largely in all the experiments at Burlington, Vermont.

A count of the stand one year later showed the following results:

Check	75 standing	moss and weeds plentiful.
Potassium permanganate	86 standing	moss and weeds plentiful.
Sulphuric acid	370 standing	moss and weeds entirely absent.

This shows an ultimate saving of four times the check, and an immediate saving of $1\frac{1}{4}$ times the check, by the use of the acid.

Another bed of *Pinus ponderosa* which had begun to damp-off was divided into thirds. Washed sulphur was applied to one plat, copper sulphate and lime to another, and the third left as a check. A count of the total stand the next spring showed the following results:

Washed sulphur	130 plants standing
Copper sulphate and lime	205 plants standing
Check	125 plants standing

Experiments were conducted in 1908 at Burlington with *Pinus sylvestris*. In the fall estimates were made by two other persons besides the writer, but no counts were made. Each bed was considered a unit and was divided into three 4 by 4 foot plats, the middle one being the check. The treatments were made soon after the seedlings came through the surface of the soil. The results are given in table I.

Owing to the early drought of 1908 the coniferous seed sown at Saranac Inn and Lake Clear Junction, New York, germinated very poorly and unevenly. This led to the virtual abandonment of the experimental beds there. The experience of past seasons had been so encouraging with sulphuric acid that this was considered the most promising chemical to use after germination. Because of the poor germination it became necessary to water the partially germinated beds. This immediately started damping-off when tried. Hence, the acid solution, at the rate of $\frac{1}{2}$ and $\frac{1}{4}$ ounce to 1 gallon of water, was used very extensively for watering such beds. Until the very last of the season this was apparently successful, but about August 1 burning developed, causing uncertainty as to the practical use of this chemical. Experiments with ammonium-copper-carbonate solution of the usual strength as a soil fungicide before sowing the seed showed that this chemical may prove to be very effective for this purpose.

In 1909 the experiments at Burlington, Vermont, were carried on with *Pinus sylvestris*.

The beds were divided into quarters by two median lines, one crosswise and one lengthwise, one plot in every bed being used as a check. One and one-half gallons of solution were applied to each treated plat. Equal quantities of seed were sown on the plats after the latter were treated.

TABLE I

Results of experiments in the treatment of damping-off in 1908 at Burlington, Vermont

TREATMENT AFTER GERMINATION	NUMBER DAMPED OFF	CONDITION IN THE FALL
Flowers of sulphur	10	Poor stand
Washed sulphur	40	Poor stand
Check	60	Good stand
Resublimated sulphur	90	Good stand
Precipitated sulphur	200	Good stand
Check	150	Poor stand
Ammonium copper carbonate (3 gal.) 1 application	...	Entirely dead
Sulphurous acid (½ oz. to 1 gal.) 3 applications	210	Better stand than check
Sulphurous acid (1 oz. to 1 gal.) 3 applications	165	Better stand than check
Check, watered 3 times	225	
Sulphuric acid (½ oz. to 1 gal.) 3 applications	60	Same as check
Sulphuric acid (1 oz. to 1 gal.) 3 applications	50	Better than check.
Check, watered 4 times	200	
Bordeaux mixture, 1 application	...	Not so good as check
Lime-sulphur (self-boiled) 1 application	60	Better than check
Check, watered once	100	
Formalin (½ oz. to 1 gal.) 2 applications	500	Entirely dead
Formalin (1 oz. to 1 gal.) 2 applications	175	Same as check
Check, watered 4 times	200	
Copper sulphate and fullers' earth (1 to 10)	80	25 per cent better than check
Potassium permanganate and fullers' earth (1 to 10)	110	Same as check
Check	100	

Table II gives the results of these experiments.

Because of the small amount of damping-off which took place, the results do not fully indicate the relative efficacy of the chemicals used. The results with formalin seem to indicate that this was not used strong enough to thoroughly kill out the damping-off fungus. It is also probable that germination was slightly inhibited and prevented in the formalin plats, although the differences in stand may possibly be explained by unavoidable variation, which is almost sure to influence results in experiments of this kind.

The ammonium copper carbonate plainly injured germination, some of the death of seedlings undoubtedly being due to the effect of the chemical, especially with the strongest solution.

Sulphuric acid probably increased germination by preventing the damping-off of weak seedlings before they came through the surface of the soil.

In 1910, experiments were started at Saranac Inn, New York, but little damping-off was apparent after the seedlings pushed through the

TABLE II

Results of experiments in preventing damping-off in 1909 at Burlington, Vermont

	DAMPED-OFF		HEALTHY		
	Single plats	Average	Single plats	Average	
				Number	Per cent
Formalin (½ oz. to 1 gal. water)	822		3060		
Formalin (½ oz. to 1 gal. water)	445	634	3564	3312	83.9
Formalin (¼ oz. to 1 gal. water)	308		3564		
Formalin (¼ oz. to 1 gal. water)	243	276	2760	3162	92.1
Formalin (⅛ oz. to 1 gal. water)	156		2988		
Formalin (⅛ oz. to 1 gal. water)	129	143	3420	3204	95.7
Check (1½ gal. water)	88		3000		
Check (1½ gal. water)	56	72	4776	3888	98.2
Ammonium copper carbonate (1 oz. copper carbonate and 1 pt. ammonia to 10 gals. water), 1½ gals. applied	501		1800		78.2
Ammonium copper carbonate, ½ above strength	158		2784		93.9
Ammonium copper carbonate, ¼ above strength	154		2520		94.3
Check (1½ gals. water)	114		3300		96.7
Commercial sulphuric acid (½ oz. to 1 gal. water)	104		4632		
Chemically pure sulphuric acid (½ oz. to 1 gal. water)	42		4068		
Chemically pure sulphuric acid (½ oz. to 1 gal. water)	55	67	4404	4368	98.5
Commercial sulphuric acid (¼ oz. to 1 gal. water)	101		4452		
Chemically pure sulphuric acid (¼ oz. to 1 gal. water)	46		4992		
Chemically pure sulphuric acid (¼ oz. to 1 gal. water)	56	68	4500	4648	98.6
Commercial sulphuric acid (⅛ oz. to 1 gal. water)	116		3792		
Chemically pure sulphuric acid (⅛ oz. to 1 gal. water)	68		3252		
Chemically pure sulphuric acid (⅛ oz. to 1 gal. water)	39	74	4884	3946	98.2
Check (1½ gals. water)	154		3732		96.0
Check (1½ gals. water)	52		4344		98.8
Check (1½ gals. water)	94		3360		97.3
		100		3812	97.4

surface. Each entire 4 by 12 foot bed was treated on May 11 with a solution as shown in the following table. On May 14 seeds of *Pinus sylvestris* were sown. On one-half of each bed untreated seeds were used, while on the other half the seeds were thoroughly wet with a solution of formalin (1 oz. to 1 gal. of water) just before sowing. The seeds were all covered with sand after sowing. The results are given in table III.

In another series of untreated beds a test was made of heavy versus light watering. The lightly-watered beds had no damping-off, while the heavily-watered beds lost about one-third of their total stand in spite of the watering being discontinued as soon as damping-off showed to any extent.

TABLE III

Results of soil and seed treatment in 1910 at Saranac Inn, New York

TREATMENT OF SOIL.	DAMPING-OFF		HEALTHY	
	Single plats	Average	Single plats	Average
Formalin (9 oz. to 9 gals. water). Untreated seed	none		600	
Treated seed	none		1450	
Sulphuric acid (18 oz. to 18 gals. water) Untreated seed	42		1400	
Untreated seed	30	36	660	1030
Treated seed	142		1400	
Treated seed	85	114	1440	1420
Check: Untreated seed (watered once before seeding)	10		900	
Treated seed (watered once before seeding)	25		800	

Besides the above specifically mentioned experiments many others of a similar nature were made, in which, because of accidents, unavoidable variations, poor germination, etc., no exact counts were made. In the earlier work the importance of exact counts was not realized. Many of the experiments upon which counts were not made corresponded in general with the more significant ones herein mentioned, so that the latter are thus rendered of even greater importance than appears on their face.

Burning with sulphuric-acid solutions apparently varies much, according to the species of seedling treated. In New York and Vermont, solutions of 1 ounce to 1 gallon of water, or one-half that strength, do not ordinarily burn the following species when only a single application is made: *Pinus sylvestris*, *P. ponderosa*, *P. strobus*, *P. resinosa* Ait. However, repeated applications of these, or even of weaker solutions, occasionally may result in burning on any or all of these species. *Picea excelsa* Link, *P. parryana*, and *P. engelmanni* are more liable to be burned.

TESTS OF MODIFICATIONS OF NURSERY METHODS

In July and August, 1906, a series of experiments were arranged out of doors at St. Louis, Missouri, to test the influence of different types of soil and various methods of management of seed beds. They were repeated in April and May, 1907, with practically identical results. So far as possible the conditions of soil, water, etc., were identical in all plats, except as stated in the following. Each bed was 4 by 12 feet in area and was raised about 3 inches above the paths. The soil was a very rich sandy loam which had been used for years as a vegetable garden, and which had been very thoroughly worked over once or twice each season, so that it was very uniform throughout the area used. In the first test seeds of *Pinus ponderosa* were used, while those of *Pinus sylvestris* were used in the second. Bed No. 1 was very heavily manured; bed No. 2 had the original soil replaced with dredged sand to a depth of over 6 inches; bed No. 3 had the original soil replaced with sterile clay subsoil to a depth of over 6 inches; bed No. 4 was watered about twice as heavily as the rest; bed No. 5 was kept as dry as possible, but frequent rains somewhat interfered with this; bed No. 6 was constantly shaded; bed No. 7 was a check; bed No. 8 had high board sides (1 foot); bed No. 9 was of garden soil upon which the seed was sown and then covered with about one-fourth inch of fine roofing gravel; bed No. 10 had two small (2 inch) tile running lengthwise about 6 inches below the surface, through which all water was applied; bed No 11 was a check. In this series the exposure, soil, etc., were so uniform that few checks were used. Equal quantities of water were given except where otherwise stated. Notes of the results were taken about one month after germination. Beds No. 3 (clay) and 9 (gravel cover) (pl. VI, fig. 4) were far better than any others and remained practically free from damping-off. In bed No. 1 (heavy manure) over 50 per cent of the stand damped-off. Bed No. 2 (sand) germinated very slowly and very poorly, but there was no damping-off. Bed No. 4 (heavy watering) lost the entire stand from damping-off. Bed No. 5 (light watering) damped-off about 25 per cent. The rains were largely responsible for this loss, as it occurred immediately after a series of daily showers. Bed No. 6 (shaded) entirely damped-off (pl. VI, fig. 1). Bed No. 7, (check) lost about 50 per cent by damping-off. Bed No. 8 (high sides) lost about 35 per dent by damping-off. Bed No. 10 (sub-irrigation) (pl. VI, fig. 2) lost over 75 per cent by damping-off. Bed No. 11 (check) lost about 35 per cent by damping-off. The freedom of beds Nos. 3 and 9 from the disease is very significant when contrasted with the heavy losses in most of the others. Bed No. 3 indicates that relatively sterile soil, such as may ordinarily be secured about a foot below the surface, aids much in the control of the disease (pl. VI, fig. 5). Prob-

ably the impervious nature of the heavy clay prevented the rapid spread of fungi. Pettis' experience (37) with the sand sub-soil with which he covers the seed, has indicated on a large scale that sterility of the soil covering the seed is a matter of considerable importance. Bed No. 9 indicates a feasible nursery practice which will be valuable under some conditions. Those species which are most susceptible to damping-off may be protected with a gravel covering at a small expense in most localities.

One commercial nurseryman[6] has been using gravel in this way for several years with fair success.

Considering the failures in the preceding tests, it appears that heavy watering, constant shade, and sub-irrigation are disastrous.

SUMMARY OF WORK ON CONIFEROUS SEEDLINGS

In conclusion it is but fair to state that the damping-off of conifers is caused by several widely-different fungi and occurs under such different conditions in different places that no rule of treatment can be devised which will apply generally. An intelligent study must be made of each locality before any statement can be made as to the best culture methods to be used for the prevention of damping-off in that locality.

The experimental work seems to lead to the following conclusions: Soil sterilization sufficient to prevent damping-off is effectively accomplished with formalin (preferably 1 ounce to 1 gallon of water, or stronger, with a suitable interval before seeding), sulphuric acid (1 ounce to 1 gallon of water, or stronger), and ammonium copper carbonate (rather dilute). No chemical is perfectly effective in stopping outbreaks of damping-off when applied after germination, sulphuric acid probably being best under most conditions. Weak formalin applied after germination is worse than useless. The fungi causing damping-off are so different in their characters and modes of attack that methods of treatment and chemicals to be used will vary according to the fungus found to be causing the disease.

The seed used undoubtedly has much to do with damping-off. Observations show that seeds which are light-weight and poorly filled, such as are usually obtained in years of scarcity, produce weak seedlings which are longer in germinating and which are very subject to this disease. A specific instance may be mentioned: Seeds of *Pinus sylvestris* in the spring of 1908 were very high-priced and of poor quality, while those of *Pinus strobus* were cheap and of very good quality. The damping-off attacked only the former. In 1907 conditions were exactly reversed and so was the damping-off on the seedlings in the same nurseries.

[6] See Isaac Hicks and Son, Trees for Long Island, Stock List, March, 1910, p. 28, for an account of this, together with half-tone of seedlings in drills thus treated.

The writer is indebted to Dr. William Trelease, formerly Director of the Missouri Botanical Garden, the Vermont Agricultural Experiment Station, the New York State Superintendent of Forests, and Mr. Henry Hicks, for their cordial aid and coöperation in carrying on experiments in their grounds and nurseries.

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PLATE VI. DAMPING-OFF EXPERIMENTS WITH PINE

EXPLANATION OF PLATES

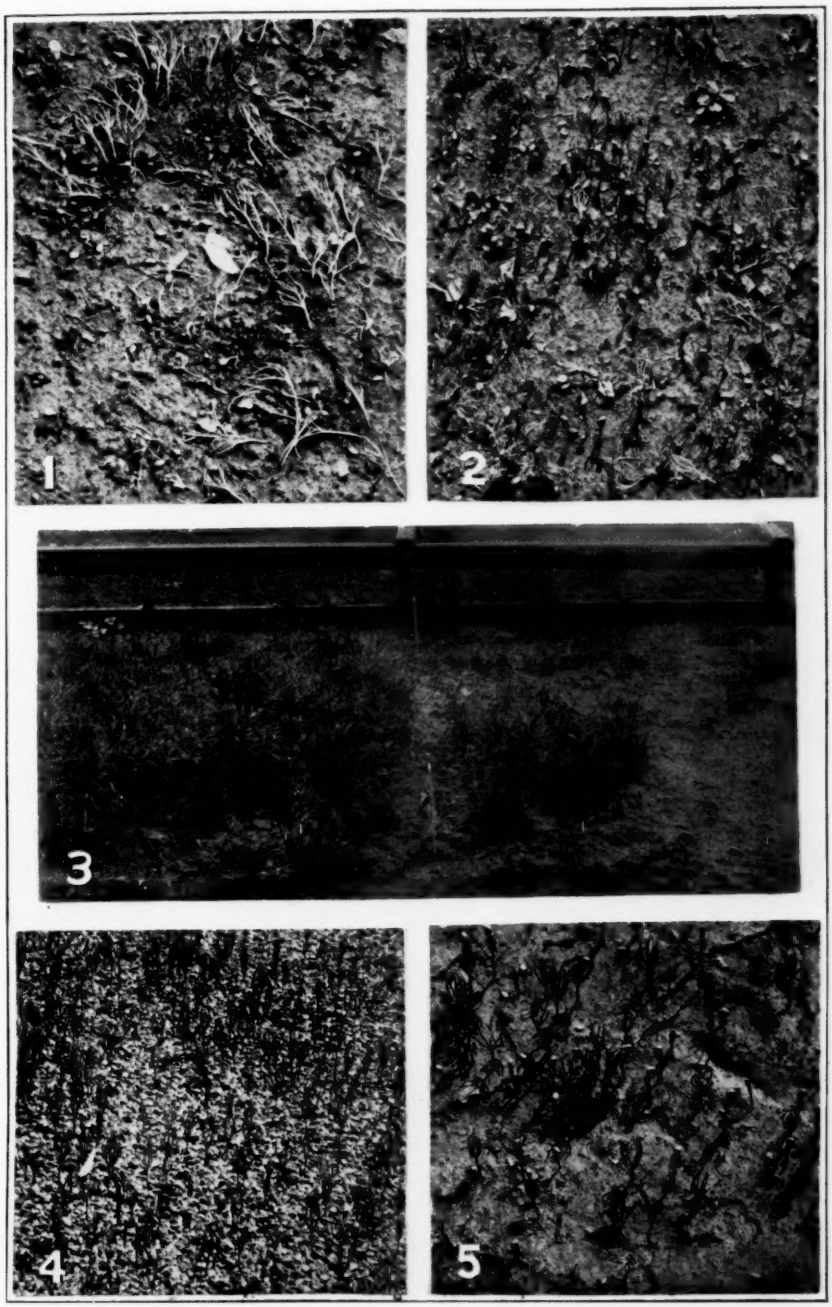
FIG. 1. Results of constant shade; all seedlings damping-off. Pinus ponderosa.

FIG. 2. Results of subirrigation; one-fourth of seedlings still healthy. Pinus ponderosa.

FIG. 3. Results of formalin soil treatment. Plat at left treated; plat at right untreated. Seedlings standing in untreated plat are evidently a result of formalin gas penetrating the soil sidewise. Pinus sylvestris.

FIG. 4. Gravel covering; resulting in perfect freedom from damping-off. Pinus ponderosa.

FIG. 5. Sterile clay; resulting in perfect freedom from damping-off. Pinus ponderosa.



SPAULDING: THE DAMPING-OFF OF CONIFEROUS SEEDLINGS.

PRELIMINARY TESTS OF DISINFECTANTS IN CONTROLLING DAMPING-OFF IN VARIOUS NURSERY SOILS

CARL HARTLEY AND THEODORE C. MERRILL

The majority of the cooperative experiments here reported were conducted during the past two seasons in the two nurseries of the United States Forest Service near Garden City, in the semi-arid portion of southwestern Kansas. The tests in 1912 were mostly carried out by Mr. J. V. Hofmann under the direction of Hartley. The nurseries are but a few miles apart, one in a fine sandy loam underlaid by clay loam, the other in a fine sand containing little humus. These will be referred to as the loam nursery and the sand-hill nursery. Loss from damping off of pine seedlings, caused mainly, as indicated by our cultures, by *Pythium debaryanum* and *Fusarium* sp., has been extremely heavy at both of these nurseries, especially at the sand-hill nursery. Fall sowing, found by the Forest Service to prevent serious losses at some nurseries, has not solved the problem for these two.

At a sand-hill nursery at Halsey, Nebraska, with soil somewhat finer-grained and better supplied with humus than that at the Kansas sand-hill nursery, $\frac{3}{16}$ fluid ounce sulfuric acid per square foot, applied in solution to beds at sowing time had been successful,[1] and four seasons of use have established its commercial value for spring sown beds. At the Kansas sand-hill nursery slightly more acid has been necessary to give results, probably because of a slightly greater alkaline tendency of the soil, and the acid has been less effective than at the Halsey nursery in preventing reinfection of the beds with damping-off parasites, though $\frac{1}{4}$ ounce per square foot has given some excellent results in spring sown beds, and $\frac{3}{8}$ ounce has done fairly well in fall sown beds. Copper sulfate, zinc chloride, and formalin have not proven successful so far in the Kansas sand-hill nursery. As at Halsey frequent watering has been necessary during the germinating period to prevent acid injury to seedlings in the spring sown beds.

At the loam nursery, sulfuric, nitric, and hydrochloric acids have failed to control damping-off, probably because of alkalinity in the soil or in the water used to sprinkle the seed beds. The water used is very alkaline to the taste. Formalin gave excellent results in some cases, but its action

[1] Hartley, Carl, Use of soil fungicides to prevent damping-off of coniferous seedlings. Proc. Soc. Am. Foresters. **7**: 96. 1912.

against parasites is too erratic to be entirely satisfactory. Mercuric chloride and ferrous sulfate have also been unsuccessful in the single series of tests made. Copper sulfate in solution at sowing $\frac{3}{8}$ ounce and more per square foot has in all cases proven advantageous in tests made with three species of pine in fall and spring sowing at this nursery. It has with one exception given stands sufficient for profitable operations. In a number of instances zinc chloride has given better results than copper sulfate and has indicated ability to stimulate germination. However, its effects are less uniform and it seems to have injured dormant seed in some plats. At this nursery $\frac{1}{2}$ ounce per square foot is probably the minimum effective strength for zinc chloride. Zinc chloride used at the time of sowing seems less likely to cause injury to germinating seedlings than equally effective amounts of copper sulfate. Present indications favor the use of copper sulfate at the loam nursery, and sulfuric acid at the Kansas sand-hill nursery. At the loam nursery it required less frequent watering to prevent injury to the seedlings by the fungicides than at the two sand-hill nurseries. In neither Kansas nursery has an entirely satisfactory treatment been developed, and tests of a variety of fungicides are contemplated for the present season.

In five other nurseries experiments outlined by Hartley have been conducted by the nurserymen. The work has been inspected and the records correlated by the writers.

At Fort Bayard, New Mexico, the experiments were conducted by Mr. H. C. Turner of the Forest Service on spring sown western yellow pine seed beds in a heavy soil. Sulfuric acid from $\frac{3}{16}$ to $\frac{5}{8}$ ounce per square foot has been tested at sowing. No injury to the seedlings has resulted from any acid treatment, and special watering seems unnecessary. Eight experimental series, extending through three seasons, show an average gain in final stand of 42 per cent on all acid plats. The stronger treatments have been on the whole a trifle more effective, but there has been little choice between strengths. Copper sulfate $\frac{1}{4}$ ounce per square foot, and zinc chloride, $\frac{3}{8}$ ounce, seemed to give good results in two series, while greater strengths gave poorer results. Injury to germinating seed is reported for even the $\frac{1}{4}$ ounce copper sulfate plat, but no injury occurred with zinc chloride. Formalin, $\frac{5}{8}$ to $\frac{7}{8}$ fluid ounce per square foot, has also been tested. In most cases it was applied two weeks before sowing. The strongest treatment ($\frac{7}{8}$ ounce) seems more than is necessary. The $\frac{5}{8}$ and $\frac{3}{4}$ ounce treatments, tested in six series during the three seasons, gave results varying from 6 per cent loss to 55 per cent gain in final stand, with an average gain of 23 per cent. Because of its greater cost and the variability in results formalin is not considered a success. Acid is now in regular use on summer

sown beds at this nursery, and will probably remain so, unless further tests show one of the salts to be consistently much more effective.

A single test of $\frac{1}{3}$ ounce sulfuric acid per square foot was made by Messrs. W. I. Hutchinson and W. H. Schrader on a fairly heavy soil in the Forest Service nursery at Monument, Colorado, in a pine bed in which loss by damping off was unusually heavy. The resulting stand was six and one-half times as great as on the untreated part of the bed. This, and other tests at this nursery in beds where damping-off was not important, indicate that here, as at Fort Bayard, special watering to prevent injury to germinating pines by acid is unnecessary.

Mr. H. D. Burrall has conducted similar experiments at the Forest Service nursery near Las Vegas, New Mexico, on a soil intermediate in texture. The species used, Engelmann spruce, is difficult to protect from parasites because of the long time during which the seedlings remain susceptible. Four plats receiving $\frac{1}{4}$ and $\frac{3}{8}$ ounce sulfuric acid at sowing showed an increase in surviving stand of 60 per cent while $\frac{3}{16}$ ounce, probably too weak, and $\frac{1}{2}$ ounce, for reasons unknown, gave an average increase of but 23 per cent over the checks. Zinc chloride, $\frac{3}{8}$ ounce per square foot, gave remarkably good results on two plats, while $\frac{5}{8}$ ounce strangely enough was a failure, again indicating the rather undependable action of this salt in soil. Copper sulfate, $\frac{1}{4}$ and $\frac{1}{2}$ ounce, and formalin, $\frac{3}{8}$ and $\frac{3}{4}$ ounce, gave little in the way of results. In no case was any injury to seedlings noted.

In the nursery of the State Board of Forestry in Vilas County, Wisconsin, a single test was made by Mr. W. D. Barnard of $\frac{3}{16}$ ounce sulfuric acid per square foot at sowing on a sandy soil. This was not followed by any serious injury to the pines. The increase in stand of 47 per cent over the adjacent untreated beds indicates a fair degree of control of damping-off, though no detailed records were made.

At the nursery of the Pennsylvania Railroad at Morrisville, Pennsylvania under the direction of Mr. John Foley, sulfuric acid was tested on a rather fine sandy loam. Smaller amounts were sufficient to cause injury here than at the Halsey nursery. The details of the injury to seedlings caused by fungicides at these two nurseries have been prepared for publication and are expected to appear soon in the Journal of Agricultural Research. The records made it possible to distinguish between acid injury and parasitic loss sufficiently to show that the acid considerably decreased damping-off. It is probable that an acid treatment much weaker than the $\frac{3}{16}$ ounce used at the Halsey nursery, followed by a watering system such as was there developed, would result in considerably increased stands at this nursery, despite the fact that losses from damping-off are ordinarily slight.

Tests of disinfectants on fall sown beds of conifers are now in progress at

two eastern and three western nurseries, and tests will probably be continued on spring sown beds in at least as many nurseries. Detailed results of work on spring-sown beds at Halsey will be published in the near future, and for other nurseries as the work in them is completed.

It seems from the work so far done that for preventing the damping-off of conifers sulfuric acid is the best disinfecting agent on most soils, as regards convenience, economy, and effectiveness. This should be applied at sowing time. It has proven more successful than heat wherever both have been tested, as it affords less chance for troublesome reinfection.

Dicotyledonous weeds which occur in the pine seed-beds have seemed much more susceptible to acid injury than have the pines. Therefore for many soils in which truck crops are to be grown, it is doubtful if the acid will be practicable for use alone. At two of the nurseries, at the suggestion of Dr. Perley Spaulding, acid disinfection has been followed by application of air-slaked lime. This treatment appears to disinfect the soil without injury to plants subsequently grown in it. But like heat sterilization it allows reinfection and because of the importance of reinfection at such places as the Kansas and Nebraska nurseries neither heat nor acid-neutralized treatments have been satisfactory. For non-alkaline soils in which heat sterilization is effective it seems very likely that equally good results can be had by disinfecting with a sufficient sulfuric acid solution a few days before sowing and then neutralizing with lime just before sowing. Sufficient neutralization should be secured in most soils by raking into the surface soil an avoirdupois ounce of air-slaked lime for every fluid ounce of acid used. For soils on which sulfuric acid is not an efficient disinfectant, zinc chloride and copper sulfate have indicated greater efficiency against parasites than formalin, and are well worth testing in general soil disinfection work.

OFFICE OF FOREST PATHOLOGY
U. S. BUREAU OF PLANT INDUSTRY

A STUDY OF THE BROWN-ROT FUNGUS IN THE VICINITY OF CHAMPAIGN AND URBANA, ILLINOIS

J. L. Conel

INTRODUCTION

Prior to 1899 the fungus which causes the disease of stone and pomaceous fruits known as "brown-rot" was considered as being of one species only, viz., *Sclerotinia fructigena*. In that year Woronin published a paper in which he states that this fungus is of two distinct species which can be distinguished from each other by marked characteristics. These species he calls *Sclerotinia fructigena* and *Sclerotinia cinerea*. He says that there is no observable difference in the mycelium of the two species, but that differences are evident in the color of the spore masses and in the susceptibility of hosts to the two forms. In *Sclerotinia fructigena* he describes the conidial tufts as light brownish-yellow or ochraceous, while in *Sclerotinia cinerea* they are invariably gray. Moreover, in the former the conidia are larger, averaging 20.9 x 12.1 microns, while in the latter they average 12.1 x 8.8 microns. Also, *Sclerotinia fructigena* occurs both on stone and pomaceous fruits, while *Sclerotinia cinerea* is found only on stone fruits.

In 1904 Aderhold found decided differences in the sizes of asci and ascospores which led him to believe that there are two species. He also observed that the ascospores of *S. frutigena* are more pointed at the ends than are those of *S. cinerea*.

Saccardo (1906) gives the measurements of asci of *Sclerotinia cinerea* as 45 to 60 x 3 to 4 microns, and of ascospores as 6 to 7 x 3 to 3.5 microns. The dimensions for the asci of *S. fructigena* he gives as 120 to 180 x 8 to 12 microns, and for the ascospores 11 to 12.5 x 5.6 to 6.8 microns.

In 1912 Voges conducted some inoculation experiments and made a distinction between *Sclerotinia fructigena*, as occurring on pome fruits, and *Sclerotinia cinerea*, on stone fruits. The results of his experiments indicate that the conidia of *Sclerotinia fructigena* lose their germinative capacity early in the winter.

Ewert (1912) made some investigations as to the manner in which the brown-rot fungi live through the winter. He accepts Woronin's views as to the existence of two distinct species. He finds that *Sclerotinia fructigena* and *Sclerotinia cinerea* differ materially in their mode of passing the winter. He thinks that the rarity of the apothecia makes it improbable

that these play an important part in maintaining the fungi. He believes that the conidia of *S. cinerea*, the species which occurs primarily on stone fruits but which can also infect pomaceous fruits, can germinate at any time during the winter in the spore cushions on the mummies of cherries, plums, and other stone fruits, and also on any pomaceous fruits that might have been infected. He finds that exposure to temperature much lower than that at Proskav, where his investigations were made, does not injure their germinative capacity. The conidia of *S. frutigena*, he finds, lose their capacity for germination very early in the winter, the fungus living through the winter solely by means of the mycelium. He also observes that the spore cushions of *Sclerotinia cinerea* are produced much earlier in the spring than are those of *Sclerotinia fructigena*. The conidia of the later are not formed at the flowering time of the stone fruits.

Jehle (1913) and Matheny (1913) made comparative studies of the fungus as found in Europe and America. Both conclude that there are two species, and that the one common to the United States is *Sclerotinia cinerea*.

Ever since Woronin divided the brown-rot fungus into two species many investigators have concluded that *S. fructigena* does not occur in the United States and that the species in this country is *S. cinerea*. One way of determining whether this is true would be for pathologists at the various colleges and universities in the country to make investigations in their respective localities. The following studies were made in the botanical laboratories of the University of Illinois under the direction of Dr. J. T. Barrett in order to ascertain whether both species of *Sclerotinia* occur in this vicinity.

STUDIES AND OBSERVATIONS

The differences suggested by those investigators who believe that the Sclerotinia which causes brown-rot is of two species may be tabulated as follows:

TABLE I

Conidial stage

	VITALITY OF CONIDIA	COLOR OF TUFTS	SIZE OF CONIDIA	DATE OF APPEARANCE IN SPRING
S. cinerea	May germinate at any time in the winter	Ashen-gray	Small 12.1 x 8.8	At the flowering time of stone fruits
S. fructigena	Lose germinating power early in the winter	Light brownish-yellow or ochraceous	Large 20.9 x 12.1	After the flowering time of stone fruits

Apothecial stage

	SIZE OF ASCI	SIZE OF SPORES	SHAPE OF SPORES	DATE OF APPEARANCE IN SPRING
S. cinerea	45–60 x 3–4	6–7 x 3–3.5	Oblong; ends blunt	At the flowering time of stone fruits
S. fructigena	120–180 x 9–12	11–12.5 x 5.6–6.8	Oblong; ends sharply pointed	

Accepting Woronin's views and assuming that the brown-rot fungus is of two species, the problems in hand is resolved to a comparison of the data in the above table with data collected by studying the fungus found in this locality.

All of the following studies were made with material from plum mummies only, since no trees other than plum were found which were infected with brown-rot.

GERMINATION OF CONIDIA

In water. Mummies Nos. 1, 2, 3 and 4 were collected from plum trees in Bronson's orchard December 9, 1912. They showed no indications of germinating conidia. Mummies Nos. 5, 6, 7, 8 and 9 were collected from plum trees in Bronson's orchard January 18, 1913, the second day of a thaw following a heavy sleet. Gentle rain had fallen the day before the collection was made. The mummies showed no signs of germinating conidia. Mummies Nos. 10, 11, 12, 13 and 14 were collected at the same time from the ground beneath the trees. These mummies also showed no indications of germinating conidia. All were one year old.

The old conidial pustules were gently scraped with a sharply pointed scalpel, care being used not to get any mycelium. The material scraped off was put into a few drops of tap water on slides, each slide being numbered to correspond with the number of the mummy. The slides were then placed in a moist chamber.

Another collection of one-year old mummies was made from the same trees on March 11, 1913. Mummies Nos. 15, 16, 17 and 18 were taken from the trees, and Nos. 19, 20 and 21 from the ground under the trees. Slides were prepared and handled as above. Mummies Nos. 22, 23, 24, 25 and 26 were taken from a tree in the horticultural grounds on April 12, 1913, and were treated in the same manner.

The slides were examined at intervals, and the results may be tabulated as follows:

TABLE II

NO. OF MUMMY	DATE COLLECTED	GERMINATION AT THE END OF				
		24 hours	36 hours	48 hours	72 hours	120 hours
1	Dec. 9, 1912			Abundant		
2	Dec. 9, 1912			Abundant		
3	Dec. 9, 1912			Absent		
4	Dec. 9, 1912			Abundant		
5	Jan. 18, 1913	*			*	
6	Jan. 18, 1913				Scant	
7	Jan. 18, 1913				Absent	
8	Jan. 18, 1913				Absent	
9	Jan. 18, 1913					Absent
10	Jan. 18, 1913	*				
11	Jan. 18, 1913	*				
12	Jan. 18, 1913	*				
13	Jan. 18, 1913					Absent
14	Jan. 18, 1913	*				
15	Mar. 11, 1913	Absent		Absent		
16	Mar. 11, 1913	Abundant		Abundant		
17	Mar. 11, 1913	Abundant		Abundant		
18	Mar. 11, 1913	Abundant		Abundant		
19	Mar. 11, 1913	*				
20	Mar. 11, 1913	Absent		Absent		
21	Mar. 11, 1913	Absent		Absent		
22	Apr. 12, 1913		Absent			
23	Apr. 12, 1913		Absent			
24	Apr. 12, 1913		Absent			
25	Apr. 12, 1913		Absent			
26	Apr. 12, 1913		Absent			

* Indicates that no conidia were on the slide.

In potato agar. A Van Tieghem mount was made as follows: A glass slide was sterilized, and a drop of sterile water was placed upon it. Material was carefully scraped into the water from an old conidial pustule on mummy No. 6. Examination under the microscope showed that conidia were present in the water, and no mycelium could be seen. With a platinum loop a drop of this water was transferred to a sterile cover glass. A few drops of sterile potato agar were put upon the drop of water on the cover glass, and the latter was inverted over a glass ring cemented to a slide. Examination under the microscope showed that a few conidia were in the agar. A check mount was made with conidia taken from a potato agar culture in petri dish No. 2.

Similar Van Tieghem mounts were made from mummies Nos. 7, 15 and 16. In the water germination tests mummies Nos. 6 and 16 yielded many

conidia which germinated, while the test with conidia from mummies Nos. 7 and 15 was negative.

Examination of the Van Tieghem mounts at the end of twenty-four hours showed the following results:

Check. All the conidia had germinated, and had formed a dense mycelium.

No. 6. Several conidia had germinated.

No. 7. None of the conidia had germinated.

No. 15. None of the conidia had germinated.

No. 16. A very dense mycelium had formed, making it impossible to follow it to its origin with certainty.

COLOR OF CONIDIAL TUFTS

On mummies. One-year old mummies were collected on April 23, 1913, from plum trees located at various points in Champaign and Urbana about one mile from each other. These points may be designated by the letters A, B, C, D, E and F. The mummies were allowed to soak in tap water for five hours, and were then put into covered glass dishes containing moist filter paper. A few tufts of conidia had appeared on some of the mummies at the end of twenty-four hours, and at the end of six days all the mummies were densely covered with conidial tufts. All of the tufts were ashen-gray in color.

On media. Plate cultures on potato agar were carefully prepared in the following manner: Sterile test tube No. 1 was filled with luke-warm, sterile potato agar, three or four drops of 50 per cent lactic acid were added, and then a few drops of sterile water containing conidia from one of the tufts on a mummy from point A. A few drops of acidulated, inoculated agar were then poured from test tube No. 1 into test tube No. 2 containing non-acidulated, sterile potato agar. The contents of tube No. 1 were then poured into sterile petri dish No. 1, and those of tube No. 2 into petri dish No. 2.

At the end of three days many colonies were making their appearance in dish No. 1, and one colony only was beginning to develop in dish No. 2. The colonies all grew in concentric zones, dense rings being developed at more or less regular distances from each other. These rings were apparently caused by a proliferation of conidiophores and conidia forming a mass which was somewhat elevated above the flat layer of conidiophores and conidia lying between and connecting the rings.

The cultures in both dishes were undoubtedly pure, and they were ashen-gray in color. Examination proved that the conidia were those of Sclerotinia.

Slant cultures were made by transferring some of the conidia from dish No. 1 to test tube No. 3 containing potato agar, and by transferring conidia from dish No. 2 to test tube No. 4 containing prune agar. A colony developed in each tube, and in both cases the color of the colony was ashen-gray. Examination showed that the conidia were likewise those of Sclerotinia.

By means of a platinum needle conidia were taken from a tuft on a mummy from point B and were put into sterile water on a sterile slide. A loop of the water was transferred to one side of sterile petri dish No. 9; part of this drop was then put on the other side of the dish. Acidulated potato agar was then poured into the dish. At the end of the second day colonies were beginning to appear, and in a few days the agar was covered with an ashen-gray culture of Sclerotinia.

In the same manner as with the mummy from point B pure cultures were prepared on potato agar with conidia taken from tufts on mummies from points C, D E and F, in petri dishes Nos. 10, 11, 12 and 13, respectively. In each culture the color of the conidial zones was ashen-gray, and the conidia were those of Sclerotinia.

Pure cultures of conidia were obtained in petri dishes Nos. 3 and 4 in a similar manner by inoculation of potato agar with ascospores from apothecia collected at point B. The conidial zones were ashen-gray in color.

In my cultural work it was clearly demonstrated that the conidiophores and conidia develop less vigorously and abundantly on prune agar than on potato agar.

SIZE OF CONIDIA

Measurements were made of hundreds of conidia both from the tufts on mummies from points A, B, C, D, E and F and from the plate cultures made from these mummies (petri dishes Nos. 1, 2, 9, 10, 11, 12 and 13), also from petri dishes Nos. 3 and 4, containing cultures developed from ascospores. In all cases the conidia were put in water on a slide and measured by means of an eye piece micrometer standardized by an accurate stage micrometer. Like Matheny, I found that variation in size was practically negligable, the mean dimensions of conidia from all the above sources being 13.3 x 7.6 microns.

DATE OF APPEARANCE OF CONIDIA AND APOTHECIA IN SPRING

Observations were made during the spring of 1913 only. The weather was neither very moist nor very dry. The tufts began to appear on the mummies at about the time the flowers of the plum opened.

The apothecia appeared on plum mummies in the spring of 1913 at about the time the plum was in full bloom. Of the large number of apothe-

cia collected all had developed from mummies older than one year. The apothecia could be found only while the flowers were on the trees, and disappeared soon after the blossoms fell. Two weeks after the fall of the petals a thorough search was made in a place where apothecia had been plentiful, but none were found.

CHARACTERS OF ASCI AND ASCOSPORES

Size. No measurements of the apothecia, asci or ascospores were made, but it was observed that the ascospores were much smaller than the conidia.

Shape of ascospores. The ascospores were ovoid in shape, with blunt ends. Pure plate cultures and slants were made on prune and potato agar from them and these were identical in manner of growth, color of conidial tufts and size with the plate cultures and slants made from the conidia taken from the mummies.

INOCULATION EXPERIMENTS

Only plum trees were found to be infected in this locality. Some inoculation experiments were conducted to ascertain whether or not other stone fruits and pome fruits may be infected by the fungus. Twigs with leaves and blossoms were sprayed with distilled water containing conidia and ascospores, and were then placed in a vessel of water under a bell-jar.

Apple. Infection from conidia seemed to be slow. At the end of the fourth day some of the petals were showing signs of infection, and a few were entirely browned. The sepals showed no indications of infection. There were no conidial pustules on any part of the flowers, leaves or twigs. At the end of the seventh day all the flower parts and many of the leaves were killed. Conidial pustules were numerous on the flower parts, as well as on the leaves and buds. Infection from ascospores seemed to be somewhat more rapid. Conidial tufts were growing on the flower parts at the end of the third day.

Crab-apple. Infection was about the same as with apple. At the end of the third day conidial tufts were growing upon the flower parts which had been sprayed with ascopsores.

Cherry. The flower parts which had been sprayed with conidia showed clear signs of infection at the end of twenty-four hours. The pistils and petals were beginning to die. Every one of the pistils was killed at the tip, and a small tuft of conidia had developed on the stigma. There were no signs of infection on the leaves. At the end of the third day many sepals were dead, tufts of conidia were on the flower parts, and some of the stems

of the flowers were killed for a few millimeters back from the flower. None of the leaves showed infection. Flowers and twigs sprayed with ascospores showed about the same conditions at the end of twenty-four and thirty-six hours as those sprayed with conidia.

Plum. Plum twigs and flowers were more easily infected than any of the other twigs and flowers. At the end of the third day all the flower parts and leaves were dead and were covered with tufts of conidia. Strands of mycelium were growing all over the flower parts. At the end of the fifth day only a few of the leaves were still green. Pustules of conidia and strands of mycelium entirely covered the flowers and leaves.

CONCLUSIONS

From these investigations I conclude that the Sclerotinia of this locality is *S. cinerea*, and for the following reasons:

1. While the conidia showed a considerable variation as to vitality, the results are nevertheless clearly positive. The fact that a considerable number of conidia germinated after passing through the winter indicates, on the basis of Voges' and Ewert's work, that the species is *Sclerotinia cinerea*.

2. The color of the conidial tufts (ashen-gray) is the same as that ascribed to the tufts of *S. cinerea* by Woronin, Matheny, and others.

3. The size of the conidia (13.3 x 7.6 microns) is essentially that reported by Woronin (12.1 x 8.8) for *S. cinerea*.

4. The date of appearance of the conidal tufts in the spring (during the flowering season of stone fruits) is unlike the condition found by Ewert in *S. fructigena* and like that in *S. cinerea*.

5. The appearance of the apothecia when the flowers of stone fruits are in full bloom, and hence in condition to be easily infected by the ascospores, as well as the persistence of the apothecia during the flowering season only, would suggest that the form here is the one which attacks stone fruits primarily, viz., *S. cinerea*. This evidence is not of much weight for the reason that some pome fruits bloom at the same time as stone fruits. Nevertheless, it is true that in two orchards apple trees which are very near severely infected plum trees show no signs of infection.

6. The shape of the ascospores is unlike that described by Aderhold for *S. fructigena* and like that described by Norton, Matheny, and others, for *S. cinerea*.

7. Finally, the fact that the form here was found only upon plum, although apple trees stand within a few feet of some heavily infected plum trees, coincides with the observations of many investigators that *S. cinerea* occurs predominantly upon stone fruits. However, as a result of my

inoculation tests, I have found that the fungus here may also infect pome fruits, at any rate under laboratory conditions, and Ewert also reports that *S. cinerea* may infect pome fruits.

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THE JONATHAN SPOT ROT

MEL. T. COOK AND G. W. MARTIN

In April, 1913,[1] the writers published a brief paper under the above title in which they expressed the opinion that the Jonathan apple spot was probably caused by a species of Alternaria. In February, 1913, Scott and Roberts[2] published a paper on this same disease in which they expressed the opinion that it was a physiological trouble.

In April, 1913, Norton[3] called attention to experiments in which he caused a spotting on Jonathan and other varieties by exposing them to formaldehyde and ammonia gas. He also said "Some badly pitted apples from cold storage had a very conspicuous ammonia-like odor in their paper wrappings, which suggested the idea that ammonia from the cooling apparatus might be a common cause of the Jonathan spot."

We stated that 90 per cent of our cultures "gave luxuriant and characteristic growth of the Alternaria" while Scott and Roberts stated that "Nearly 400 cultures of the diseased spots have been made in various ways and on various media, but no organism has been isolated with any degree of consistency. A species of Alternaria often occurred in cultures from fruit grown in the eastern part of the country, but cultures from northwestern-grown fruit were almost entirely barren. A few apparently successful inoculations were made by spraying Alternaria spores on Jonathans kept in moist chambers and the fungus reisolated, but both Jonathan and Esopus (Spitzenberg) are so susceptible to the disease that they are apt to become spotted under any conditions outside of cold storage. In some cases both the inoculated fruit and the controls contracted the disease at about the same time. Spores of this fungus inserted through needle punctures failed to produce the disease. As Alternaria is very commonly associated with rotting apples, especially when the fruit is placed in cellar or basement storage, the possibility of its being the cause of this disease becomes very remote."

Believing that there was not sufficient evidence for the confirmation of either of the above theories, the writers decided upon an entirely different

[1] Cook, Mel. T. and Martin, G. W., The Jonathan spot rot. Phytopathology **3**: 119, 120. 1913.

[2] Scott, W. M. and Roberts, J. W., The Jonathan fruit-spot. U. S. Dept. Agric. Bur. Pl. Ind. Circ. 112: 11–16. 1913.

[3] Norton, J. B. S., Jonathan fruit spot. Phytopathology **3**: 99, 100. 1913.

line of investigation, but, owing to reasons hereafter stated, decided not to publish the results. However, numerous inquiries have induced us to publish this statement of all the data in hand, believing that it may be of service to some of several workers who are interested in this problem.

In 1913 we bagged a large number of apples at intervals throughout the growing season, believing that, if the disease was due to an Alternaria, we could protect the fruit from infection and thus produce clean apples, while, if due to physiological causes, the apples in the bags would develop the spots the same as those that were unprotected. The trees were sprayed with lime-sulphur for scale before the opening of the buds, again for scab before the opening of the flower buds and again for scab and codling moth after the falling of the petals. The first bagging followed this spraying and therefore these fruits received no other treatments. Some of the fruits which were bagged later in the season received the regular additional summer treatments with self-boiled lime-sulphur. The bags were made of "glassine" paper which was semitransparent or translucent and slightly water proof. These were fastened by first wrapping cotton about the twig above the fruit and then tying the neck of the bag firmly at this point. This prevented injury to the twig and the entrance of spores from the outside. No attempt was made to sterilize the fruit or twigs before covering them with the bags and, of course, it is recognized that some spores were probably inclosed with the fruit. The first lot of about three hundred apples was bagged on May 9 and lots of two to four dozen were bagged at frequent intervals thereafter until August 15. A very large number of those bagged early in the season dropped from natural causes; while in the case of those remaining, as well as of those bagged later in the year, so many of the bags were injured by hail and rain storms that it is not possible to announce any definite conclusions. The fruit was picked October 3; part put in cold storage and part brought directly into the laboratory for immediate observation. Records were made November 13, 1913, and January 8, 1914. Although the loss of apples due to storms was so great that we hesitate to draw conclusions, a few statements can be made.

1. The number of spots on apples bagged previous to June 9 was much less than on those bagged after that date.

2. The relative injuries due to spotting may be expressed in the following incomplete table:

DATE OF EXAMINATION		APPLES WITHOUT SPOTS	APPLES WITH FEW SPOTS*	APPLES WITH MANY SPOTS
		per cent	*per cent*	*per cent*
November 13, 1913	Bagged apples	72	18	10
November 28, 1913	Unbagged apples	21	47	32
January 8, 1914 (Part of same fruit as inspected November 13, 1913)	Bagged apples	42	39	19

* Apples with ten or less spots.

It will be noted that the bagged apples were in far better condition than those that were not bagged. This may be considered a fair representation of the results obtained. If the spot is physiological, it appears that we might reasonably expect approximately as much spotting on the fruit in the bags as on that not in bags. On the other hand, the fact that we did not disinfect fruit or twigs before bagging makes it possible that some spores were imprisoned in the bags and were the cause of the spotting.

3. The smaller, nearly black, typical "Jonathan" spots are more commonly confined to the dark area of the skin.

4. The rather large, light-brown "Alternaria" spots are more common on the lighter area of the skin.

5. There are spots occurring on any area which are hard to classify as one or the other of the above and which grade into one or the other. These are not so common as the clearly distinguishable spots.

6. Many of the "Jonathan" spots are surrounded by a light-brown area characteristic of the "Alternaria" spots.

During the fall and winter of 1913–1914, numerous laboratory studies of these spots have been made. The surface of apples has been disinfected with formaldehyde or corrosive sublimate, washed in sterilized water and plate cultures made from the spots. The peel was removed from the large spots by means of a sterilized blade, care being taken not to allow the blade to come in contact with the underlying tissue, and this tissue was transferred to agar plates. The very small spots were removed and placed with the flesh side against the agar. In the former we have had as high as 93 per cent Alternaria. From the extremely small spots in which the skin only was used we found lower percentages of Alternaria, in one case as low as 30 per cent, but it must be remembered that these spots included many blackened lenticles, many of which would probably never have developed into spots. In this connection we may say that there appears to be some correlation between this disease and the size of the lenticles. We have a quantity of material which we are sectioning and

which we hope will throw some light on this phase of the problem. Checks prepared by placing healthy tissue on agar plates remained sterile.

Inoculation of diseased tissue under the peel of the apple very readily produced an Alternaria rot, while checks prepared by putting healthy tissue from lenticles under the peel of the apple did not produce the rot. The spraying of the Alternaria spores on the surface of the apple did not give satisfactory results. We could have no assurance whatever that the apples used for these experiments had not been previously infected with fungus. Needle point inoculation gave a spot very similar to, but not exactly the same as, the natural Jonathan spot. These spots usually developed very quickly into soft rots. However, it must be remembered that, if the spots are due to Alternaria, the natural infection occurred when the fruits were immature and developed with the ripening, while our needle point inoculations were made on ripened fruits.

In connection with the possibility of the production of spots by gas we will say that spots which we have described frequently occur on the fruit in the orchard, especially if it is allowed to remain on the tree rather late in the season. The apples which we used for our work were stored in closed dry glass chambers in our office for use in the early part of the season and in a cold cellar for use later. We have no reason to believe that either lot was affected with gas, certainly not with the gases used in the ordinary cold storage plant. However, having found two distinct but similar spots on the Jonathan, we are inclined to believe it probable that the investigators of this problem may be working with different spots.

In conclusion we wish to say that we fully appreciate that the Alternarias are very widely distributed and that the possibilities of infection of a physiological spot by an Alternaria are very great. We also wish to say that we do not believe that there is sufficient data for affirmation of either of the two tentative theories as to the cause of this disease. We publish our data at this time in order that it may be available for other workers.

RUTGERS COLLEGE
NEW BRUNSWICK, NEW JERSEY

THE IDENTITY OF THE ANTHRACNOSE OF GRASSES IN THE UNITED STATES

GUY WEST WILSON

A very common and wide-spread grass inhabiting fungus currently known as *Colletotrichum cereale* Manns has frequently come to the attention of the writer. That a species so wide-spread and so conspicuous should have escaped the notice of taxonomists until within the last five or six years has been the cause of considerable surprise. It was, therefore, with no small interest that the collections at the New York Botanical Garden were examined with a view to determining the true status of this species. After a cursory examination of Saccardo's Sylloge and of this material it appeared to be worth while to study the problem more seriously. In publishing *C. cereale* the reference to past literature is embodied in the following quotation:[1] "In the study of an organism such as this one, systematic difficulties are met with. Systematic mycologists in the past have not covered precisely all the points that prove to be essential in generic distinctions. This has resulted in the description of certain organisms in such a way as to be referable to any one of two or more genera. Such difficulties are met in the genera Colletotrichum (Melanconieae), Vermicularia (Sphaeropsideae) and Chaetostroma (Hyphomyceteae) . . . . . This anthracnose partakes more closely of the genus Colletotrichum, hence the organism has been provisionally thrown into this genus and given the name *Colletotrichum cereale*, n. sp."

The results of the studies of the available specimens of grass anthracnose are given in detail under the various names under which the specimens were found in the herbarium.

1. *Dicladium graminicolum* Cesati

This species, which is the type of a new genus, was described from Vercelli, Italy, on stems of *Echinochloa Crus-galli* and *Zea Mays*. Cotype material was exmained. The conidia and setae are the same as those of *Colletotrichum cereale*, the former averaging about 20 x 4μ, while the latter are about 115μ long. No other material so named was seen. The species was transferred by Saccardo to the genus Steirochaete.

[1] Selby and Manns, Ohio Agr. Expt. Sta. Bul. 203, pp. 206, 207. 1909.

2. *Psilonia apalospora* Berk. & Curt.

This name was given to material issued in exsiccati by Ravenel, on dead stalks of *Zea Mays* from South Carolina. No diagnosis was given to accompany the specimens. As the specimen at hand had reposed on an unprotected page without an envelope for over half a century the setae were so broken that they could not be measured, but the acervulus has the structure of *Colletotrichum cereale*. The conidia measure 20 to 23 x 3 to 4μ. This is well within the limits of variation of the Colletotrichum in question. Saccardo[2] pointed out the identity of this species with *Colletotrichum lineolum*. Other specimens so named were examined on *Zea Mays* (New Jersey, West Virginia) and on *Panicum sanguinale* (New Jersey). The former host appears only in the form of dead stalks, while the latter was evidently living material.

3. *Vermicularia culmigena* Cooke

This species was also issued undescribed by Ravenel on *Tripsacum dactyloides* from South Carolina. The material is a Colletotrichum with conidia and setae well within the limits of size and form of those of *C. cereale*. *Vermicularia culmigena* Desmaz. is a true Vermicularia which is found in Europe on Dactylis and Arrhenatherum.

4. *Vermicularia Holci* Sydow

Described from Germany on *Holcus lanatus*. Only cotype material was seen. This is a Colletotrichum with very small acervulae and conidia about 19 x 3.5μ. It is certainly not distinct from the species under consideration.

5. *Vermicularia Lolii* Roum.

Described from France on *Lolium perenne*. This a Colletotrichum with conidia showing a much greater range in size than the original description of 22 to 26 x 5.6μ would indicate. While no other material except the cotype was seen the species is certainly not distinct from *Colletotrichum cereale*. However those mycologists who recognize "microspora" and "macrospora" varieties would probably give it subspecific rank.

6. *Vermicularia Melicae* Fuckel

Described from Europe on *Melica uniflora*. The original description is not sufficiently explicit to differentiate the species, and as no specimen

[2] Michelia **2**: 375. 1881.

named by Fuckel was at hand its exact status cannot be determined. The material from Germany on *Melica uniflora* and *Calamagrostis epigeum* which was distributed by Sydow belongs to *Colletotrichum cereale*. The conidia average about 20 x 3.5μ.

7. *Vermicularia sanguinea* Ellis & Halsted

This species is based on material collected at Ames, Iowa, by Dr. B. D. Halsted, on a cultivated species of Panicum of European origin. The original spore measurements of 20 to 22 x 6μ is entirely too large, as the type specimen shows a range of 18 to 22 x 4 to 6μ. While the spores are a trifle broad in proportion to their length the discrepancy does not appear to be sufficient to warrant its retention as a distinct species. The chief distinction appears to be the reddish margin of the infected area, which may be due rather to the chemical composition of the leaf than to a difference in the parasite. Other specimens so named are on *Panicum Crus-galli* (Kansas) and on *Sorghum* sp. (Alabama). The species was later transferred to Colletotrichum.

8. *Vermicularia graminicola* Westend.

The cotype material of this species which was examined was not in a condition to warrant an opinion as to the identity of the fungus, as no acervulae were found. However other European material issued by Allescher and Schnabl and by Roumeguere under this name is a true Vermicularia. American material which was placed under this name, but which is in reality identical with *Colletotrichum cereale*, was examined on *Anthaenantha rufa* (Florida), on *Cenchrus tribuloides* (Delaware), and on *Poa* sp. (New Jersey).

9. *Vermicularia affinis* Sacc. & Briard

The type of this species was collected in France on some undetermined species of grass. As no authentic material was at hand for study its identity has not been established, but the description would indicate a true Vermicularia. Material so named from Mississippi on both *Andropogon Hallii* and *Panicum Crus-galli* is a Colletotrichum which agrees in every respect with *C. cereale*.

10. *Vermicularia* sp.

Undetermined material so labeled on *Dactylis glomerata* (Mississippi) and on *Arundinaria* sp. (Alabama) evidently belong to the species of Colletotrichum under consideration. While setae and conidia agree in

size, the conidia from Arundinaria are in the main somewhat less curved than is usual in *C. cereale*. The difference, however, does not appear to be sufficient to warrant the separation of this form as a distinct species.

11. *Colletotrichum lineola* Corda

This species was first described from dead Umbelliferous stems, but later almost any species of Colletotrichum with elongate acervulae was referred to it. As a result *C. lineola* "Aut." is a species of astonishing variability in conidial and other characters, to say nothing of its extreme diversity of hosts. As Corda's figure is such as to give no warrant for supposing that his fungus and the grass-inhabiting forms which have been so named are the same species it is impossible to include this name among those of the grass anthracnose. In the specimens on grasses which have been referred to this species the conidia average 20 to 22 x 2 to 4μ, and the setae about 100 to 150μ long. This places the material among that which must be considered as belonging to *Colletotrichum cereale*. The material examined is listed below:

On *Andropogon nutans* (Kansas), *Festuca heterophylla* (France), *Festuca* sp. (Italy), *Panicum virgatum* (Louisiana), *P. Crus-galli* (Louisiana), *Sorghum saccharum* (Connecticut, New Jersey, France) *Sorghum* sp. (Alabama), and *Zea Mays* (New Jersey).

12. *Colletotrichum Bromi* Jennings

This species is known only from the type collection on *Bromus unioliodes* in Texas. It is merely a form of *Colletotrichum cereale* with small sporodochia, but otherwise typical.

13. *Colletotrichum lineola pachysperma* Ellis & Kellerm.

The fungus so named on *Chrysopogon nutans* from Kansas has conidia which measures 20 to 30 x 4 to 7μ. While its other characters would indicate that it is identical with the species described by Manns, the extreme size of the conidia will appeal to some as entitling it to the dignity of at least a trinomial. However as the forms considered as identical in this paper have a range of conidial measurements of 18 to 26 x 3 to 6μ this one does not materially change these measurements. In case a separate name is desired for the large-spored form, Roumeguere's *Vermicularia Lolii* appears to be the earliest published name for such a fungus. As the present name merely appeared on exsiccati labels, it cannot be considered as properly published.

14. *Colletotrichum cereale* Manns

Material under this name distributed by the Ohio Experiment Station has been examined on all the hosts mentioned at the time of original publication, except *Hordeum vulgare* and *Triticum spelta*. In addition similar specimens on *Arrhenantherum elatinus* and *Agrostis alba* have been examined. All agree with each other in their characters and show no points of difference from the material which has been discussed in the preceding pages.

All of these forms, which occur either on living or dead grasses, agree in habit and in the size and form of both conidia and setae, the measurements with few exceptions falling within a reasonable range of variation for a species which is so widespread both geographically and in its hosts. It would appear then that we are dealing with a single wide-spread species, of which possibly a large-spored form should be recognized. Were material of other grass-inhabiting species of Vermicularia and Colletotrichum at hand for comparison it is not impossible that some of these would be added to this already long list of names which have been applied to the species in question.

The synonomy and a recapitulation of the specimens examined, as well as other similar data is appended. The hosts are arranged by tribes. It is interesting to note that eight of the twelve tribes of the family are represented.

Colletotrichum graminicolum (Cesati) nom. nov.

Dicladium graminicolum Cesati, in Rabenh., Herb. Viv. Myc. *1677*. Flora **35**: 398. 1852.

Psilonia apalospora Berk. & Curt., in Ravenel, Fungi Carol. Exs. **3**: 83. 1855. (Hyponym)

Vermicularia culmigena Cooke, in Ravenel, Fungi, Amer. Exs. *531*. 1881. Not *V. culmigena* Desmaz., Ann. Sci. Nat. III, **3**: 363. 1845.

Steirochaete graminicola Sacc. Syll. Fung. **4**: 316. 1886.

Vermicularia sanguinea Ellis & Halst., Jour. Myc. **4**: 8. 1888.

Colletotrichum lineola pachyspora Ellis & Kellerm., in Ellis & Ev. N. Am. Fungi *2181*. 1889. (Hyponum)

Colletotrichum Bromi Jennings, Texas Agr. Expt. Sta. Bul. 9: 25. 1890. (Hyponym)

Vermicularia Lolii Roun., Rev. Myc. **13**: 131. 1891.

Colletotrichum sanguineum Ell & Hals.; Ellis & Ev. N. Am. Fungi *3466*. 1896.

Vermicularia Holci Sydow, Hedwigia (Beibl.) **38**: 137. 1899.

Colletotrichum cereale Manns, Selby and Manns, Ohio Agr. Expt. Sta. Bul. 203–207. pls. 1–10. 1909.

Specimens examined (arranged by tribes of Gramineae)

On Maydeae:

Tripsacum dactyloides L., South Carolina (Ravenel.).

Zea Mays L., Connecticut (Rorer), New Jersey (Ellis), South Carolina (Ravenel), West Virginia (Nuttall); Italy (Cesati).

On Andropogoneae:

Andropogon Hallii Hack., Mississippi (Tracy).

Chrysopogon nutans (L.) Benth., Kansas (Bartholomew, Kellerman *927*, Kellerman and Swingle).

Sorghum saccharum L., New Jersey (Ellis), France (Roumeguere).

Sorghum sp. (Jerusalem corn), Alabama (Underwood).

Sorghum sp. (Kafir corn), Alabama (Lamson-Scribner *10*).

On Paniceae:

Anthaenantia rufa (Ell.) Schult, Florida (Curtis *142*).

Brachiaria digitarioides (Carpenter) Nash, (*Panicum Curtisii* Chap.), Louisiana (Langlois).

Cenchrus tribuloides L., Delaware, (Commons *2571*).

Echinochlos Crus-galli (L.) Beauv. (*Panicum Crus-galli* L.), Kansas, (Bartholomew), Louisiana (Langlois *841*), Mississippi (Tracy, White).

Panicum virgatum L., Louisiana (Langlois *324*).

Panicum sp. cult., Iowa (Halsted).

Syntherisma sanguinale (L.) Dulac (*Panicum sanguinale* L.), New Jersey (Ellis).

On Agrostideae:

Agrostis alba L., Ohio (Selby).

Calmagrostis Epigeos (L.) Roth, Germany (Sydow).

Phleum pratense L., Ohio (Selby)

On Aveneae:

Arrhenantherum elatinus (L.) Beauv., Ohio, (Selby).

Avena sativa L., New Jersey (Schwarze), Ohio (Selby).

Holcus lanatus L., Germany (Sydow).

On Festuceae:

Bromus secalinus L., Ohio (Selby).

Bromus uniolioides (Willd.) HBK., Texas (Jennings).

Dactylis glomerata L., Mississippi (Tracy), Ohio (Selby).

Festuca heterophylla Lam, France (Faurey).

Festuca sp., Italy (D. Saccardo).

Melica uniflora Retz., Germany (Sydow).

Poa pratense L., Ohio, (Selby).

On Hordeae:

Lolium perenne L., France (Foutrey).

Secale cereale L., Ohio (Van Meter).

Triticum vulgare L., Ohio (Selby).

On Bambuseae:

Arundinaria sp., Alabama (Earle).

DISTRIBUTION: Southern New England to Iowa and Texas; also in Western Europe.

Exsiccati:

As *Colletotrichum lineola* Corda.

Briosi & Cavara, Fungi Par. *374*, Ellis, N. Am. Fungi *816*, Ellis & Everh., Funghi Columb. *154*, Roum, Fungi Gall. *1777*, Roum. Fungi Sel. Exs. *7008*, D. Sacc. Myc. Ital. *117*.

As *Colletotrichum lineola pachyspora* Ellis & Kellerm.

Ellis, N. Am. Fungi *2183*.

As *Colletotrichum sanguinea* Ellis & Halst.

Ellis & Everh., N. Am. Fungi *3466*.

As *Dicladium graminicolum* Corda.

Rabenh. Herb. Viv. Myc. *1677*.

As *Psilonia apalospora* Berk & Curt.

Ellis, N. Am. Fungi *54*, Ravenel, Fungi Carol. Exs. 3: *82*.

As *Vermicularia culmigena* Cooke.

Ellis, N. Am. Fungi *741*, Ravenel, Fungi Amer. *531*.

As *Vermicularia Holci* Sydow.

Sydow, Myc. March. *4846*.

As *Vermicularia Lolii* Roum.

Roum. Fungi Gall. *5775*.

As *Vermicularia Melicae* Fuckel.

Sydow, Myc. March. *2455*, *4848*.

THE PLASTER CAST APPLE SPECIMEN

LEO. E. MELCHERS

WITH ONE FIGURE IN THE TEXT

It is not an easy matter to preserve diseased specimens of apple, pear, quince etc., so as to maintain the characteristic appearance of the fungus and the natural color of the fruit. It has been the writer's experience that the great majority of preserving fluids, and I believe most of them have been given a trial by me, are not satisfactory when one wishes to keep a diseased apple or pear specimen in its natural diseased condition. Good results can be obtained when absolutely healthy and sound fruit is preserved, by proper manipulation of the right chemical solutions, but the diseased fruit preservation problem is quite another thing. For example, those who have made an attempt to preserve apples affected with Baldwin spot disease, have probably found that the characteristic appearance (the depressed spots) change. Instead of the surface remaining pitted with these sunken areas, it assumes a smooth appearance. This is brought about by the absorption of preserving fluids and its striking appearance is lost.

A method with which the writer has experimented and finally found entirely successful in keeping specimens of apple affected with some of our common diseases, is as follows. Any of our superficial apple troubles, such as scab, blotch, cedar apple rust, the different "spots," sooty and fly speck fungus, etc., can easily be kept for years in their natural condition by employing what I call the plaster of Paris "cast." A spherical segment bearing the fungus is cut from the apple. I find that a segment about the size of a silver dollar is generally the most practical, but I have often used half an apple with good results (figure 1). The pulp is carefully removed by means of a scalpel. This is best accomplished by making criss-cross cuts into the pulp, being carefully not to puncture the skin. It is then an easy matter to remove the pulp, as it comes out in small pieces. All the flesh should be taken out and finally the inner skin (collenchyma) should be lightly scraped to remove the last fragments.

When this has been accomplished, the mere shell remains, the apple skin and its diseased area. The necessary materials for the completion of this apple "cast" are, plaster of Paris, a spatula, and a small saucer. The plaster of Paris is made into a soft paste (using water) and this filling is put

into the mould by means of the spatula. One should be careful not to allow the plaster of Paris to come in contact with the surface of the segment bearing the fungus, as it may prove difficult to remove. After the skin mould is filled up level, the specimen is finished and should be laid aside and allowed to set. This requires ten or fifteen minutes. The skin adheres tightly to the cast and will not alter its shape.

In using this method the exact form of the segment is retained and the appearance of the fungus is not altered. It is quite surprising to find that the skin of the apple does not shrink, loosen, nor change color. I have specimens of scab, blotch, spot, spray injury, etc., on apple which are three years old, and they look as natural as the day they were cut from the fruit. It is to be remembered, however, that these "casts" should be handled with

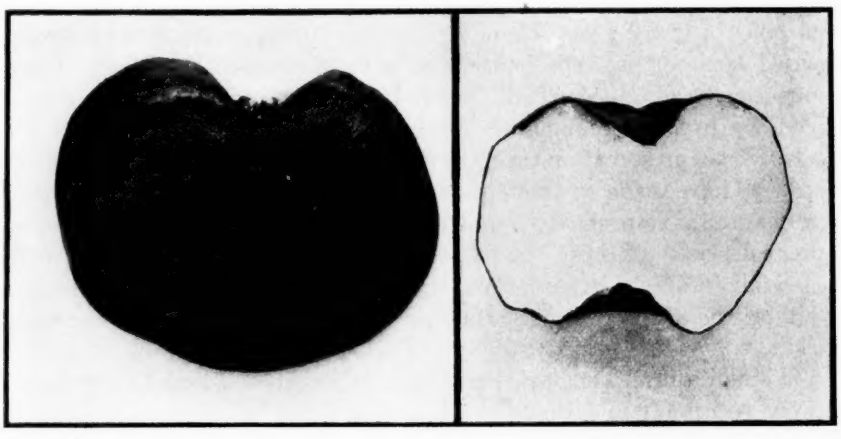

FIG. 1. Photographs of two plaster cast specimens of apple blotch disease, the one at the right showing the back side with plaster filling.

moderate care and ought not to be exposed to direct sunlight. I have them in Riker specimen mounts which protect them from injury, and when not in use they are kept out of the direct sun-light. This prevents the skin from fading.

It will pay those who are teaching plant pathology or who are engaged in institute work, to give this simple, inexpensive method a trial. Although I have not tried it on vegetables, it appears as if it could be effectively used on those having a fairly tough skin. Furthermore, the writer sees no reason why this method could not be employed by the horticulturist in building up a collection of normal type specimens.

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A METHOD OF PICKING UP SINGLE SPORES

C. W. Edgerton

With One Figure in the Text

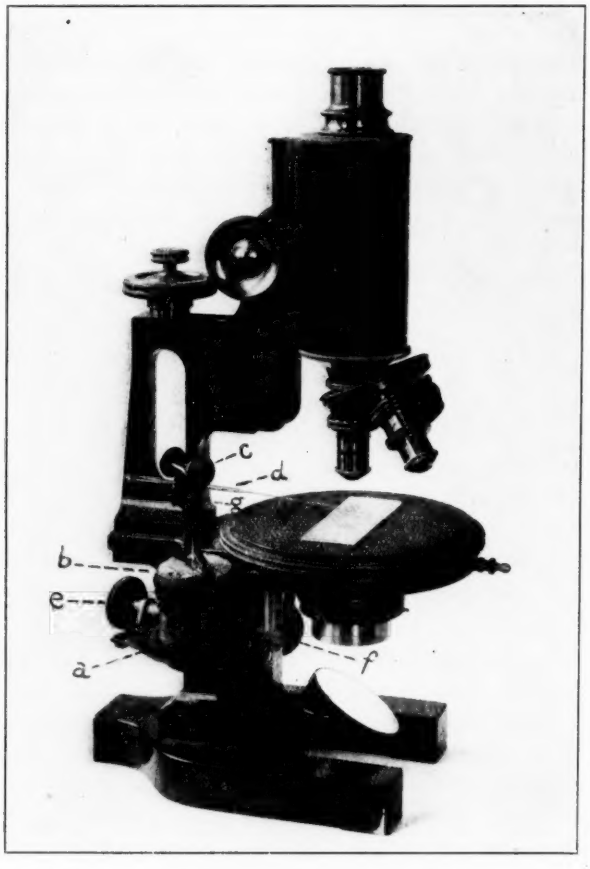
Frequently in mycological or pathological work it is necessary to isolate single spores or asci and transfer them to culture media where their development can be watched. This is particularly important in cases where but few asci are present and these are mixed with many conidia or loose ascospores. In order to make sure that a colony really develops from an individual spore or ascus, it is necessary to pick this out of the mixture and transfer it to the desired culture medium.

During the past two years, while working on cultures of Glomerella, an apparatus was devised which gave fairly good results. The principle involved is not new, as similar devices have been used by Barber and others, but the simplicity of the apparatus may appeal to other workers along this line.

A Bausch and Lomb microscope of the DD type was used. The lower diaphragm under the condenser was thrown out to the side (*a*), and a large cork (*b*) wras cut and fit tightly into the opening. Into a groove in the top of this cobk was placed an ordinary rubber tube clamp (*c*) and this was held in place y a rubber band around the cork. A very small tube of 2mm. inside diameter was then drawn out to a very fine point. After sealing the large end, this tube (*d*) was placed in the clamp between two pieces of cork (*g*) so that the end of the capillary tube came between the objective of the microscope and the slide on which the material to be examined was placed.

The mechanisms which regulate the substage of the microscope give all desired motions to the capillary tube. The whole apparatus, cork and all, turn readily giving a transverse motion of the tube across the field, while the screw (*e*) which regulates the angle of light when the diaphragm is under the condenser forces the capillary tube forward or back. When the point of the capillary tube is in the position desired it can be lowered into the preparation and again lifted out by the screw (*f*) which raises and lowers the condenser. The spore or ascus is drawn into the tube or forced out by the contraction or expansion of the air in the closed end of the tube. Ether and a small gas flame are useful to produce the contraction and expansion of the air. A little ether dropped on the upper end of the tube will cause a contraction of the air, sucking in the object from the slide, and it can be forced out again by a little heat.

In the work with the Glomerella cultures, in which the single asci were scattering and the loose ascospores and conidia abundant, an ascus was picked up from the preparation and then forced out on a sterile cover slip. The very small drop on the cover slip was then examined with the microscope to make certain that only the ascus was present. If this was found to be the case, the whole cover slip was pushed down into a petri dish



containing agar until the drop was just covered. If there were other spores carried over with the ascus, the cover slip was either discarded or else the ascus was again picked up. After the ascus is transferred to the petri dish, it can be watched during germination to make doubly sure that no other spores are present.

A number of capillary tubes should be provided in advance and these should be placed in plugged test tubes and sterilized. They are then ready

for use at any time. These capillary tubes should be made from as small glass tubes as possible, not larger than 2 mm. inside diameter. In a smaller tube, there is a less volume of air and the expansion and contraction of this is less, making the manipulation easier. Also a smaller tube can be drawn out more satisfactorily than a larger one.

LOUISIANA AGRICULTURAL EXPERIMENT STATION

PHYTOPATHOLOGICAL NOTES

Tylenchus dipsaci in the United States. Mr. L. P. Byars' paper on this pest read at the Atlanta meeting, makes it seem desirable to record in a more accessible place, my discovery of this nematode in 1907. I received early in that year, a report from Edgerton, Kansas from a farmer whose rye field was suffering from some unknown trouble, and from the specimens submitted, determined the trouble to be due to this pest. On visiting the field in the latter part of that summer, large areas in his field were found to have the plants very badly stunted and in many cases killed. An examination of the stems showed the very characteristic dwarfing and an abundance of nematodes within the tissues.

Similar nematodes, apparently of the same species, were received from Charlestown, South Carolina, causing injury to oat plants. It is probable that this pest has been introduced into this country many times and will be found widespread when looked for. The presence of this nematode was reported in the Year Book of the U. S. Department of Agriculture for 1908, page 582, in the Summary of Plant Diseases by W. A. Orton and Adeline Ames, but owing to a misunderstanding was listed under the beet instead of under rye.

ERNST A. BESSEY

Resistance in tobacco to hydrocyanic acid gas injury. During January, 1914, four varieties of tobacco were being grown at the University of Wisconsin on greenhouse benches in separate rows in various parts of the house. When the plants were approximately 6 inches high the house was fumigated to destroy the white fly, using approximately ½ ounce of potassium cyanide per 1000 cubic feet of space. Under the particular conditions present the gas caused a striking injury to three varieties, namely, Little Dutch, Cuban, and White Burley, as shown by a discoloration and wilting of the leaves. The variety, Connecticut Broadleaf, remained free from injury in all parts of the house, showing a striking resistance to this form of injury. No satisfactory explanation has been formulated to account for this difference in susceptibility to hydrocyanic acid gas injury. The fact is simply made a matter of record as of interest in connection with the fundamental questions of variation in disease resistance.

JAMES JOHNSON

REVIEWS

Opredielitel gribov. Jaczewski, A. A. von. 2^e isdanie. Tom I. str. I–XXII+1–934. Griby soviershennyie. Departament Zemledielia S. Peterburg. 1913. (Classification of Fungi. Jaczewski, A. A. von. 2nd. ed. Vol. I, pp. I–XXII+1–934. Fungi Perfecti. Published by the Department of Agriculture, St. Petersburg. 1913.)

Until a few years ago very little was known about the subject of phytopathology in Russia, and only a few specialists were interested in mycological matters. Even now the general public and practical farmers do not realize the economic importance of this branch of science in spite of great annual losses from fungous diseases.

Within the last fifteen or sixteen years the amount of attention given to this subject has been rapidly increasing. Several mycological laboratories have been established, a number of popular and technical publications issued, experimental and demonstration work organized, all of which is now being carried on by a score or two of instructors scattered over the country. A pioneer and leader in this work is A. A. Jaczewski, Director of the Phytopathological Institute of St. Petersburg. The first edition of his work on the classification of fungi, which was the first book of the kind to appear in that country, was published in 1897. At that time the Russian mycological flora was so insufficiently explored that only the descriptions of genera were given.

A great change has taken place since that time. Thousands of specimens which have been collected and preserved in several modern herbaria show that there are about 6000 species of fungi within the boundaries of the Russian Empire, or about ten per cent of the total number known. It is interesting to note that there is a characteristic difference between the mycological flora of Russia and that of Middle Europe, in that it also contains certain American forms. With this abundant material at hand, Professor Jaczewski has recently revised and greatly enlarged his work so that it will now consist of two volumes, the first of which has recently appeared.

The classification adopted by the author is based on the general principles worked out by Lotsy, although one essential change is made in this system by establishing a special group of Mesomycetes. These are characterized by the formation of sporangia like the lower fungi, and by the development of a septate mycelium, as is the case with the higher fungi.

This should not be confused with the Mesomycetes of Brefeld, who so designated the intermediate stages in the development of reproductive organs from sporangia to asci or basidia. The latter theory is not accepted at all by Professor Jaczewski and he states quite positively that according to the new cytological researches there is no such relation between sporangium on one side and, ascus or basidium on the other.

All perfect fungi are divided into four large groups, or classes, which are as follows: A, Myxochytridineae Gobi.; B, Phycomycetes De Bary.; C, Mesomycetes Jacz. (nec Brefeld); D, Mycomycetes Brefeld (including Ascomycetes and Basidiomycetes).

Among Myxochytridineae are descirbed Plasmodiophoraceae Zopf., Monolpidiaceae A. Fischer, and Merolpidiaceae A. Fischer. A somewhat similar group, Myxomyceteae, will be described by the author in the second volume of his work and will include those amoeboidal organisms which always live upon the surface of the substratum in saprophytic conditions only.

Class Mesomycetes is a small one, and, although the author believes that further study of this group will give sufficient material to form several families, at the present time he includes here only one family with the following seven genera: Mycelophagus Mangin, Protomyces Unger, Taphridium Lagerheim & Juel., Dekkenbachia Jaczewski, Aphanistis Sorokin, Ascoidea Brefeld, and Basidiobolus Eidam.

The classification of the other groups is more or less that generally accepted with the exception of Pyrenomycetes, which are grouped according to the author's own system, published in 1894 (Jaczewski, Essai de classification des Pyrenomycetes. Paris. 1894).

The book has several other features which may be of a special interest to American mycologists. Thus, for example, the boundaries of the genus Peziza as well as the genus Polyporus are laid very wide. Peziza is divided into ten subgenera and includes at least 435 species. Polyporus is subdivided in four subgenera (Poria Pers., Fomes Fries., Polystictus Fries., and Eu-Polyporus Jacz.) and includes more than 1800 species. The writer is unable to go into further details on account of space limitations but since so few American are able to read the original work in Russian it is hoped that Professor Jaczewski himself will contribute an article to an early number of Phytopathology, which will embody the essence of his system of classification.

The volume which has appeared contains 610 excellent illustrations, which include reproduction of photographs and drawings and one colored plate, illustrating fourteen chief colors used in description of fungi.

A copy of Doctor Jaczewski's book will be sent upon request to any of the mycological laboratories in America. The present address of the

author is: Bureau of Mycology and Phytopathology, English Perspective 29, St. Petersburg, Russia.

MICHAEL SHAPOVALOV

Bitter Pit Investigation. The past history and present position of the Bitter Pit question. McAlpine, D. First progress report 1911–12. By authority: A. J. Mullett, Acting Government Printer, Melbourne.

The trouble of pome fruits known in English as bitter pit, or fruit pit, in German as "Stippen" and in French as "Points bruns de la chair," has for many years been known to apple growers in various parts of the world. In Canada it is not uncommon, and is widely distributed geographically, but while exacting its annual toll of loss, it can hardly be considered one of our most serious orchard troubles. In some parts of the world, however, notably South Africa and Australia, the injury caused by it is very severe, and in the last-named country it was judged to be of such importance that Mr. D. McAlpine, the well known plant pathologist, was in 1911 appointed by the federal government to give his undivided attention for a term of years, or until a solution shall be obtained, to the problem of its nature and control. To plant pathologists in general the disease is of interest from the fact that, while it is generally conceded to be of non-parasitic origin and associated most probably with variations in sap-pressure, the hypotheses put forward to explain the mechanism of the process are very diverse; while to American pathologists it is of additional interest since it is probable that the term "Baldwin spot" is commonly applied indiscriminately to two distinct diseases, one being the physiological trouble in question and the other due to a parasitic fungus *Cylindrosporium pomi* Brooks. Renewed interest was given to the subject by the publication in 1911 of papers by two Australian investigators, Dr. Jean White[1] and Prof. A. J. Ewart[2] in which they endeavored to show that Bitter Pit was the result of the poisoning of the pulp cells by the mineral poisons used in spraying, and possibly also by poisons absorbed from the soil.

Mr. McAlpine's first report on the work undertaken since his appointment now lies before us in the form of a handsome quarto volume, containing some 200 pages of text and descriptive letter press, and 35 plates with 133 illustrations of great excellence, mostly from original photographs. The work here summarized includes a detailed histological study of the pome fruits with special reference to the vascular system; the characteristics of

[1] White, Jean., Bitter pit in apples. Proc. Roy. Soc. Victoria, N. S. **24**: 1–19. 1911.

[2] Ewart, A. J., On bitter pit and the sensivity of apples to poisons. Ibid., pp. 367–419. 1911.

the disease; a critical review of the literature on its history and distribution and the hypotheses advanced to explain it; an analysis of the replies to a series of questions submitted to growers regarding especially the contributing factors; and an outline of experiments carried out and in progress.

A form of the disease described under the name of "crinkle," or confluent bitter pit, which is characterized by the surface of the fruit being thrown into rough folds with large cavities in the underlying tissue, is new to us. According to the author it has apparently been recorded, outside of Australia, only from California.

As regards the causes of the trouble, Mr. McAlpine considers that the evidence is entirely in favor of the hypothesis that it is due to irregularities in the factors influencing the balance between transpiration and water supply, and not to poisoning of cells, e.g., by arsenical sprays. Both the recorded history of the disease and its presence on unsprayed trees are against the latter hypothesis. It was also found experimentally that fruit on an unsprayed tree, protected from any possible contact with spray material from other parts of the orchard by being enclosed in calico bags as soon as the fruit had set, was quite as badly affected as the exposed fruit of the same tree. The author sums up the matter of poisoning from the exterior as follows:

"After testing the effects of various chemical substances applied to the skin of the apple, I cannot emphasize it too strongly that all this production of external spots and smears has nothing to do with Bitter Pit. This disease originates from within, and the action of an external agent on the skin is something totally different."

The principal contributing factors are given as follows:

1. Intermittent weather conditions when the fruit is at a critical period of growth.
2. Amount and rapidity of transpiration.
3. Sudden checking of the transpiration at night when the roots are still active owing to the heat of the soil.
4. Failure of supplies at the periphery of the fruit followed by spasmodic and irregular recovery.
5. Irregularity of growth, so that the vascular network controlling the distribution of nutritive material is not regularly formed.
6. Fluctuations in temperature when fruit is in store.
7. Nature of variety.

The question of storage conditions is particularly important, since the disease usually develops to a large extent in storage. It was found, however, that "even with very susceptible varieties the development of Bitter Pit was retarded by keeping them at an even temperature of 30° to 32° F." It is recommended "that the apples should be picked . . . just when

they have reached their full size, and on the green side, and placed in cold storage without delay."

We congratulate Mr. McAlpine on the work he has already accomplished, and shall look forward with increased interest for the results of his experiments directed towards the control of the trouble in the growing fruit.

J. W. EASTHAM

ANNOUNCEMENT

At the Atlanta meeting of the Phytopathological Society a committee was appointed to arrange, if possible, for the preparation and publication of a complete list of current Pan-American phytopathological literature which may in part take the place of reviews. Through the courtesy of the U. S. Department of Agriculture and especially Dr. Wm. A. Taylor, Chief of the Bureau of Plant Industry, an arrangement has been effected by which the list prepared by the Librarian of the Bureau of Plant Industry, Miss E. R. Oberly for the department files is to be supplied to this journal. The first installment of this list will appear in the next number of PHYTOPATHOLOGY. It is hoped to have the list include all phytopathological literature of any value published in North and South America; also the West India Islands and the islands under the control of the United States beginning with January, 1914. This is a large undertaking and can only be successfully accomplished by the most active and thorough coöperation of every member of our Society. It is earnestly requested that each member send abstracts of all phytopathological papers to Miss Oberly and also advise her of any omissions or errors that may be noted in the published lists. It is deemed best to omit anonymous publications.

The committee urges that each member do all possible to promote the success of this new feature of our Journal.

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